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THE EFFECTS OF BETA-ANTAGONIST AND EPINEPHRINE INFUSION ON WARM- AND COLD-ACCLIMATED SHEEP

BY

COLEEN MARY HARMAN

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
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DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

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Coleen Harman

Permanent Address:

407 Silverthorn Way, N.W.

Calgary, AB T3B 4E8

Date: August 21, 1992

UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis titled THE EFFECTS OF BETA-ANTAGONIST AND EPINEPHRINE INFUSION ON WARM- AND COLD-ACCLIMATED SHEEP submitted by COLEEN MARY HARMAN in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

R.J. Christopherson (Supervisor)

J.R. Thompson

L.C.H. Wang

Date: August 19, 1992

DEDICATION

To the memory of my mother and friend,

MARY BAILEY STEWART HARMAN

who taught me to never give up,

even at the bitter end.

ABSTRACT

In this study, the hypothesis investigated was that catecholamines, acting on β-adrenoceptors, are responsible for a portion of the increase in heat production due to cold exposure, and that inhibition of β_1- and β_2-adrenoceptor subtypes has differential effects on heat production and plasma metabolites in a cold environment. In the first experiment, six shorn yearling wethers acclimated to 26°C (WA) and six acclimated to 2°C (CA) were fed, once daily at 16:00 h, a diet of alfalfa hay (midbloom, ME = 8.8 MJ kg^{-1} and 12% crude protein) at levels of 1.2 and 1.5 kg d^{-1}, respectively. On different days sheep were given infusion treatments of saline (control), a β_1-antagonist (metoprolol), or a β_2-antagonist (ICI 118,551), at rates of 2.5, 5.0, and 10.0 $\mu g\ kg^{-1}\ min^{-1}$ between 09:00 and 15:00 h. In the second experiment, two WA animals and two CA animals from the first study were exposed to 26°C and infused with saline, epinephrine (0.3 $\mu g\ kg^{-1}\ min^{-1}$), and epinephrine with metoprolol or ICI 118,551 (5.0 $\mu g\ kg^{-1}\ min^{-1}$). Cold exposure significantly increased ($P<0.05$) heat production (from 0.210 to 0.486 kJ $kg^{-0.75}\ min^{-1}$), glycerol (0.213 to 0.432 mg dL^{-1}), glucose (51.88 to 60.56 mg dL^{-1}), plasma urea nitrogen (16.66 to 23.25 mg dL^{-1}), valine (177 to 333 nmol mL^{-1}), isoleucine (96 to 128 nmol mL^{-1}), leucine (108 to 189 nmol mL^{-1}), and norepinephrine (3.262 to 14.22 pmol mL^{-1}) concentrations, and significantly decreased ($P<0.05$) glutamine (343 to 258 nmol mL^{-1}) concentrations in plasma. Decreases in heat production due to β-blockers only approached significance ($P<0.09$), but β-blockers did reduce glycerol concentrations ($P<0.05$), both during cold acclimation and epinephrine infusion, indicating that the rate of lipolysis was

decreased. The medium dose of β_2-blocker increased plasma glutamine and branch-chain amino acid concentrations ($P<0.05$). However, the decrease in glycerol concentration did not always correlate to a decrease in heat production or increase in plasma amino acid levels. Both β_1- and β_2-antagonists exerted minor effects on HP, but they exerted more pronounced effects on indices of fat mobilization, with a more consistent response shown by the β_2-antagonist. The results of this study indicate that the β-adrenoceptor effects of catecholamines may play a role in the increase in heat production of sheep during cold exposure.

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INTRODUCTION

Catecholamines and β-adrenoceptors have been implicated in contributing to an increase in heat production (HP), for the maintenance of body temperature during cold exposure. Endogenous catecholamines are believed to cause a portion of the increase in HP during cold exposure (Webster et al. 1969b). Additional support for this has been the demonstration of an increase in plasma epinephrine (E) and norepinephrine (NE) levels, with a concurrent increase in oxygen consumption, and hence an increase in HP in cold-acclimated animals (Christopherson et al. 1978). The β-adrenoceptors must play some role in this increase in HP, due to an approximate 10% decrease in cold-acclimated HP in sheep upon the administration of propranolol, a β-adrenergic blocker (Christopherson and Kennedy, pers. comm.).

Nonspecific β-blockers or β_1-blockers have been used in past studies with ruminants, but the effects of β_2-blockers on HP in domestic animals have not been extensively examined. The reason to look specifically at β_1- versus β_2-blockers, is to determine if the type of tissue whose energy substrates are used by the body to generate heat can be altered in some way. In addition, better characterization of the adrenergic receptors responsible for stimulating HP in ruminants is necessary. It is unknown if it is possible to spare the body's protein tissues at the expense of fat stores for HP. Perhaps there could be preferential selection of tissues by catecholamines for thermogenic substrates, due to the predominance of β_1-adrenoceptors on adipocytes, and β_2-adrenoceptors on skeletal myocytes (McDowell and Annison 1991).

The objectives of this study are to determine whether β_1- and β_2-adrenoceptors play differential roles in induction of changes in HP and associated metabolic responses in warm- and cold-acclimated yearling wethers, and to determine whether HP can be selectively supressed to conserve energy without jeopardizing thermoregulation and protein anabolism. There is some indication that cold-exposed sheep can maintain their rectal temperature even with a decrease in HP upon the administration of propranolol (Webster and Hays 1968).

LITERATURE REVIEW

The ability of an animal to survive in a cold environment, for instance, during the winter months, is determined by the animal's ability to minimize the cold stimulus and by its ability to respond to that stimulus (Webster 1974a). Webster (1974b), described different receptors for cold stimuli which exist in the skin, hypothalamus, spinal cord and abdominal viscera. The effects of acute exposure of an animal to a cold environment include stimulation of shivering in striated muscle, increase in heart rate, increase in depth of respiration, and in general, activation of sympathetic and pituitary endocrine systems such that the rates of biological oxidation in all tissues are elevated.

METABOLIC RESPONSES TO COLD

Cold-induced thermogenesis is the increase in HP above thermoneutral metabolism, when an animal is exposed to temperatures

below its lower critical temperature (Webster 1974a; Sasaki and Weekes 1986). Cold-induced thermogenesis consists of both shivering and nonshivering thermogenesis (NST), and is controlled by somatic and autonomic nervous systems, and neuroendocrine influences. Induction of cold-induced thermogenesis is presumably related to an increase in activity of the sympathetic nervous system (SNS) (Graham et al. 1980), as the apparent release of NE has been shown to increase in adult dogs during acclimation to 3°C (Crist and Rosmos 1987), and in other domesticated animals under cold stress (Thompson et al. 1978c; Graham et al. 1981). In addition, plasma E levels have been shown to be increased in animals exposed to chronic cold (Christopherson et al. 1978; Graham et al. 1981). In human patients, it has been shown that treatment with β-adrenergic antagonists (e.g., propranolol, metoprolol) reduces resting metabolic rate (Fagher et al. 1988; Welle et al. 1991). By measuring oxygen consumption in animals, it is possible to estimate HP (McLean 1972). Oxygen consumption has been shown to decrease in domestic animals, following the use of β-adrenergic antagonists (Christopherson and Brockman 1989). This suggests that β-adrenergic activity makes a contribution to resting energy expenditure.

Shivering thermogenesis in ruminants is believed to be the major response to acute cold exposure, while NST is the predominant adaptive response to chronic cold exposure. Shivering thermogenesis is possibly a less efficient process than NST, because increased air movement, created by shivering, will reduce external insulation and heat generated in the external musculature will be lost rapidly (Sasaki and Weekes 1986).

Thermoregulatory NST is a mechanism for producing heat by means other than shivering, whose sole function is to maintain homeothermy in a cold environment. Webster (1974b) stated that NST only existed in those species that have significant amounts of brown adipose tissue (BAT). In BAT mitochondria, the carrier protein thermogenin has been proposed as an important mechanism for H^+. This protein reduces the coupling of oxidative phosphorylation from electron transfer by decreasing the mitochondrial proton gradient. The result is energy dissipation without the production of ATP (Newsholme and Leech 1983).

Many forms of NST exist; including basal metabolic rate, the thermogenic effect of food, and the thermogenesis associated with pregnancy, lactation, and exercise. However, the latter are not considered specifically thermogenic, although they may serve as useful heat sources in cold environments. Presently, it is believed that NST is also produced in other tissues, because NST is observed in animals, such as ruminants, that have little or no BAT as adults (Thompson et al. 1978b; Graham et al. 1981). As well, Sasaki and Weekes (1986) have stated that ruminants such as sheep are not often observed shivering during chronic cold exposure. This suggests that ruminants become more dependent on NST to maintain body temperature. Consequently, it is of interest to discover the mechanisms and substrates used for NST in adult ruminants during cold exposure.

Sheep do not always show evidence of skeletal muscle shivering during chronic cold exposure, even though blood flow to muscles is strongly stimulated. During chronic cold exposure, there is a transition period where shivering thermogenesis is progressively

substituted by NST. For both shivering thermogenesis and NST in sheep, muscle has been suggested as the primary site of cold-induced thermogenesis due to the increase in blood flow to non-diaphragmatic skeletal muscles during chronic cold exposure (Schaefer et al. 1982). Schaefer and coworkers (1982) recommend that myoelectrical measurements should be used to more accurately assess muscle tone, and thus distinguish between shivering and NST.

Since skeletal myocytes do not receive direct sympathetic innervation, yet skeletal myocytes possess adrenoceptors, they must be stimulated by circulating catecholamines (mainly E) from the adrenal medullae. Norepinephrine is present in the blood, but is there primarily as spill-over from tissues where neural release occurs. An example of this release is from the sympathetic nerves innervating skeletal muscle blood vessels (Rodger and Bowman 1983). Norepinephrine is quickly removed and degraded by the enzymes monoamine oxidase and catechol methyl transferase (Newsholme and Leech 1983), or removed by reuptake by sympathetic nerve endings (Hadley 1984).

Ruminants adapt to chronic cold exposure in various ways in order to reduce their lower critical temperature. The objective is to minimize the impact of low effective ambient temperature, which is the integrated impact of all environmental factors (e.g. temperature, wind, rain) on an animal's ability to maintain internal homeothermy. The impact of effective ambient temperature is minimized by increasing resting metabolism, body insulation, and food intake. When food intake is held constant during constant cold exposure, the metabolic responses measured must be distinguished from the consequences of alterations in

energy balance as a result of cold thermogenesis (Sasaki and Weekes 1986).

Graham and Christopherson (1981) have demonstrated a calorigenic response (i.e. an increase in oxygen consumption, and thus HP) of sheep to E which was potentiated by chronic cold exposure. Catecholamine-induced NST may occur in ruminant skeletal muscle since neither NE or E were observed to induce shivering. However, there is some evidence that E can facilitate shivering by enhancing neural pathways involved in shivering (Robertshaw 1977). Young (1975) and Schaefer (et al. 1982) have also shown that increased thermoneutral HP in cattle and sheep is associated with decreased visually-assessed shivering intensity after prolonged periods in the cold. Further studies are required to test the theory that perhaps overt shivering is simply replaced by a less perceptible increase in muscle tone (Sasaki and Weekes 1986). Gregg and Milligan (1982) have shown that a major contribution to cold thermogenesis could be from elevated plasma membrane sodium-potassium ATPase pump (Na^+,K^+-ATPase) activity in skeletal muscle. More studies are also required to determine the possible roles of thyroid hormones in regulating the amount of Na^+,K^+-ATPase, and catecholamines in regulating the activity of this enzyme (Sasaki and Weekes 1986).

SUBSTRATES FOR THERMOGENESIS

Ensuring delivery of adequate supplies of substrates and oxygen to thermogenic tissues is a primary objective during cold exposure, so concurrent increases in whole-body oxygen consumption and cardiac output

are evident during cold stress (Thompson et al. 1975). Since exposure to cold is a type of stressor, many of the classic "flight or fight" responses are evident, due to stimulation of the sympatho-adrenal axis. Norepinephrine is released at sympathetic nerve endings, while E (predominantly) and NE are secreted from the adrenal medullae into the circulation. The catecholamines cause a reduction in blood flow to the viscera, while increasing blood pressure, heart rate, cardiac output, and blood supply to skeletal muscles (Hadley 1984; Guyton 1991). Acute cold stress greatly increases blood flow to shivering muscles, via dilation of their blood vessels by catecholamines, whereas chronic cold exposure increases blood flow to the thyroid gland and the non-diaphragmatic skeletal muscle (Schaefer et al. 1982).

During acute cold exposure of adult ruminants, skeletal muscle shivering is the major source of cold-induced thermogenesis (Webster 1974a). The substrates for shivering are provided initially by muscle glycogen and triacylglycerol (TAG) reserves. As these stores are depleted, other substrates are used for energy metabolism (Herpin et al. 1987). Adipose tissue and intramuscular fat depots are mobilized to provide free fatty acids (FFA) for beta-oxidation and the tricarboxylic acid cycle, and glycerol for gluconeogenesis. The bovine hindlimb switches from a net output to a net uptake of FFA, as the turnover of FFA increases (Sasaki and Weekes 1986). Glucose turnover, as a result of hepatic glycogenolysis and gluconeogenesis, and glucose oxidation have also been shown to increase in sheep following acute cold exposure (Thompson et al. 1978b). The liver has also been shown to increase uptake of the glucose precursors, propionate (Thompson et al. 1978a) and

glycerol (Thompson et al. 1975). However, there has not been evidence of significant hepatic uptake of lactate and glucogenic amino acids during acute cold exposure in ruminants (Thompson et al. 1978b). There is very little data on the change in blood substrate concentrations, such as glycerol, plasma urea nitrogen (PUN), and amino acids, following chronic cold exposure. If there were consistent findings available on these parameters, it would be easier to assess the effects of different temperatures on the energy substrates used for HP, as well to enable researchers to determine whether their animals' responses to their cold-acclimation protocol is too extreme for the health and welfare of the animals.

Interpretation of the literature concerning metabolite levels during chronic cold exposure is difficult due to differences in experimental protocol. For example, different researchers have used a variety of temperatures, duration of cold exposure, age, weight, and fleece depth for sheep. Animals fed the same in a cold environment, as they are in a warm environment, may be in a negative or reduced energy balance, whereas levels of glucose precursors increase when feeding level increases in the cold (Sasaki and Weekes 1986). Increases in both FFA turnover and glucose turnover may increase HP due to the heat produced from substrate cycles (Newsholme and Crabtree 1976). Weekes et al. (1983) have shown that glucose turnover increased by 40% after 7-21 d of cold exposure at 0°C, at a fixed level of intake. McKay et al. (1974) allowed sheep to increase food intake with cold exposure at -2°C for 6 wk, and recorded a 150% increase in glucose turnover.

Plasma FFA concentrations have been reported to increase (Thompson 1977; Bell and Thompson 1979) or remain unchanged (Graham and Phillips 1981) during chronic cold exposure, however, plasma FFA concentrations also depend on the level of food intake (Horton 1981). Free fatty acids make a contribution to thermogenesis, as indicated by a reduced respiratory quotient in cold-acclimated sheep. McKay et al. (1974) estimated that glucose only accounts for about 10% of the energy substrates used for whole body thermogenesis. Tsuda et al. (1979) also found a similar percentage contribution of glucose to HP. An increase in glucose oxidation in the cold could result in an increased demand for amino acids as glucogenic precursors, unless adequate amounts of propionate and other substrates are available from the diet. Tsuda et al. (1979) also reported that propionic acid, glutamic acid, alanine, and glycerol made the same percentage contributions to glucose synthesis at both 20°C and 0°C in ewes. Therefore, some increase in the use of all these substrates would occur in the cold. Conversely, Early et al. (1990) obtained results which suggested that the rate of alanine conversion to glucose increases during chronic cold exposure. However, they propose that the longer time period (21-25 d) of cold exposure may have contributed to the activation of the Cori cycle. In another study, Tsuda et al. (1984) found that the percentage of heat derived from acetate oxidation decreased, but that from FFA's increased markedly at 0°C and that these two substrates together accounted for 50% of the total HP.

ENDOCRINE RESPONSES TO COLD

Acute cold exposure increases sympatho-adrenomedullary activity, which in turn increases lipolysis, glycogenolysis, and gluconeogenesis. Sasaki and Weekes (1986) have concluded that catecholamines, therefore, increase substrate supply to thermogenic tissues, rather than having a direct calorigenic effect. In addition, glucocorticoids have a permissive function, which enhances the mobilization of substrates. Sasaki and Weekes (1986) proposed that acute cold exposure results in a net catabolic response to increase the mobilization of substrates for shivering thermogenic muscle. They also propose that cardiovascular changes, rather than direct hormonal effects, stimulate increased substrate uptake and oxidation by the musculature. However, Newsholme (1986) believes that catecholamines may also have a direct calorigenic effect, by increasing the activity of substrate cycles.

Plasma NE and E concentrations are increased during chronic cold exposure (Christopherson et al. 1978), and urinary catecholamine excretion has also been found to increase under these conditions (Sasaki and Takahashi 1980). Cold acclimation of shorn sheep at mild temperatures (8-13°C) has been shown to potentiate the calorigenic response to exogenous E, but not to NE (Graham and Christopherson 1981). These conditions have also been shown to potentiate the hyperglycemic response to E in fed sheep (Graham and Phillips 1981). This implicates catecholamines as having a role in the increase in HP during cold exposure, in order to maintain body temperature. Past research has been inconsistent in the amount of time between feeding and the recording of

HP in both acutely and chronically cold-exposed animals, and this may lead to misleading results due to the thermic effect of food, as well as the anticipatory response of animals to the time of day they are fed. Both of these factors may cause an increase in HP (Webster 1983).

Thyroid hormone levels may potentiate the responses to catecholamines in chronic cold, depending on the duration of cold exposure and the level of feed intake. Increased thyroid hormone levels may increase skeletal muscle mitochondrial number and activity, and thus increase the thermogenic capacity of skeletal muscle (Sasaki and Weekes 1986).

Panaretto and Vickery (1972) reported that severe acute cold stress increases plasma cortisol concentrations in sheep. The influence of chronic cold exposure on plasma cortisol concentration has not been established, however it has been shown that prolonged constant, but not intermittent cold exposure causes an increase in cortisol metabolic clearance rate in sheep (Graham et al. 1981). It has been suggested by some authors (Webster 1974a; Himms-Hagen 1983) that cortisol and the thyroid hormones have permissive and direct effects on the response to catecholamines. This potentiation allows tight regulation of the response of the animal to cold exposure, allowing an enhanced rate of glucose and FFA turnover to be maintained, supplying energy for shivering thermogenesis and NST in skeletal muscle, and also enabling the animal to rapidly respond to any further increases in the intensity of cold exposure (Sasaki and Weekes 1986).

In response to elevated sympatho-adrenomedullary activity and thyroid hormone concentrations, the reduced level of insulin secretion

in a cold environment allows enhanced mobilization of glucose and FFA. However, insulin concentrations are still large enough to allow replenishment of glycogen stores when nutrients are available (Sasaki and Weekes 1986).

CATECHOLAMINE RECEPTORS

The role of catecholamines in controlling body metabolism during cold exposure is a challenging area of study because of the ubiquitous location of catecholamine receptors (adrenoceptors) and their diverse effects on all body tissues (Hadley 1984). Since the discovery of the different α- and β-adrenoceptors that catecholamines act upon (Ahlquist 1948), and their subtypes (α_1, α_2, β_1, β_2, and recently β_3 (Arch 1989)), researchers have tried to separate the effects of stimulation of each of these adrenoceptors. Some α- and β-adrenoceptors are found on the same cell types, for example, vascular smooth muscle cells, however, they may not be found in equal numbers. This allows for tight regulation of tissue functions and rapid reaction to extracellular stimuli. Some cells may have a predominance of one receptor, for example, β_1-adrenoceptors on white adipocytes, with a very small amount or absence of the other receptors (Swenson 1984).

The α-adrenoceptors tend to have more of an effect on physical attributes such as blood pressure and glandular secretion, as well as presynaptic negative feedback on NE secretion (McDowell and Annison 1991). The β-adrenoceptors also affect these functions, but usually in opposition to the α-adrenoceptor effects. In addition, β-adrenoceptors

are found on various tissues such as adipose tissue and muscle, and the stimulation of these receptors affects the mobilization of energy substrates in these tissues (Fain and Garcia-Sainz 1983; Swenson 1984). There are relatively few studies on the location, concentration, and activity of these receptors in ruminant tissues. This information would be useful in determining if ruminant responses to catecholamines are significantly different than monogastric responses.

β-adrenoceptors exert their metabolic effects by initially increasing adenylate cyclase activity in the cell, which then increases the intracellular cyclic adenosine monophosphate (cAMP) concentration. Stimulation of β_1-adrenoceptors stimulates lipolysis and increases metabolic rate, whereas stimulation of β_2-adrenoceptors increases glycogenolysis in liver and muscle, increases protein accretion, and stimulates presynaptic NE production (McDowell and Annison 1991).

Norepinephrine is described as being the classic neurotransmitter catecholamine, due to its local effects post-synaptically, with very small amounts of NE being found in the blood to exert effects distal to its site of release. In contrast, E has classic hormone qualities, as E acts on tissues proximal and distal to its site of secretion, after transport through the blood. Both NE and E have very short half-lives in the blood, as they are degraded within minutes of release. This allows rapid deactivation of any response to these catecholamines, once secretion has ceased (Newsholme and Leech 1983; Hadley 1984).

Norepinephrine has a greater affinity for, and a greater ability to stimulate α-adrenoceptors, while E has a greater effect on β-adrenoceptors, but can also act on α-adrenoceptors (Hadley 1984).

Therefore, E infusion has been used to study β-adrenoceptor functions (Graham and Christopherson 1981; Graham and Phillips 1981). In addition, β-adrenergic agonists and antagonists have been used alone, and in conjunction with E, to elucidate the metabolic effects of stimulating β_1- and β_2-adrenoceptors (Holloway et al. 1985; Thornton et al. 1985; Stock and Rothwell 1986; Christopherson and Brockman 1989).

CELLULAR RESPONSES TO CATECHOLAMINE STIMULATION

Epinephrine and NE affect cells by a very similar mechanism. Catecholamines binding to a plasma membrane receptor activates a guanosine-triphosphate (GTP)-binding protein, which then interacts with adenylate cyclase to cause a change in the concentration of cAMP. This then changes protein kinase A activity. Protein kinase A is an enzyme which phosphorylates several key enzymes, for example, hormone-sensitive lipase in adipose tissue, with a concurrent change in enzyme activity. Stimulation of either β_1- or β_2-adrenoceptors causes activation of adenylate cyclase, via stimulation of a GTP-binding G_s protein (also known as N_s). When catecholamines stimulate the α_2-adrenoceptor, another GTP-binding protein, the G_i protein (also called N_i) is activated, which in turn inhibits adenylate cyclase. Catecholamines can also stimulate α_1-adrenoceptors, which activate the phosphoinositide system, but this has not been studied in ruminant animals (Vernon and Sasaki 1991).

Cold exposure and seasonal effects can elicit changes in the responsiveness of tissues to hormones. In many cases, this is due to a

change in receptor number, but a change in receptor number does not always result in altered responses. There may be changes in maximal response, in sensitivity (concentration of a hormone required to produce half-maximal effect), or a combination of both of these factors. Changes in hormone responsiveness may also be due to changes in receptor affinity or through modification of the signal transduction systems following hormone binding. As a result of these adaptations, changes in hormone concentration may not necessarily reflect a change in effect. Alterations in the responsiveness of tissues to hormone binding can be tissue-specific and therefore have a key role in nutrient partitioning, or homeorhesis. In general, for a wide range of hormones, it appears that ruminant cells use the same mechanism of hormone action as cells from other species (Vernon and Sasaki 1991).

SUBSTRATE CYCLES AND CATECHOLAMINES

Metabolism is regulated by very specific and very sensitive mechanisms. Sensitivity in metabolic regulation is the quantitative relationship between the relative change in enzyme activity and the relative change in concentration of the regulator. Substrate cycles are a type of mechanism for improving sensitivity of an entire metabolic pathway (Hers 1976; Newsholme 1976). Substrate cycles have also been implicated in contributing to thermogenesis in different tissues. These include: fructose-6-phosphate/fructose-1,6-bisphosphate (Newsholme 1976), TAG/FFA turnover (Brooks et al. 1982, 1983; Challiss et al.

1984a, 1984b), acetate/acetyl-CoA (Jessop et al. 1990), Cori cycle (Astrup et al. 1989), and protein/amino acid turnover (Newsholme et al. 1987).

A substrate cycle is defined as a reaction that is non-equilibrium in the forward direction of a pathway (i.e. A to B), to be opposed by a reaction that is non-equilibrium in the reverse direction of the pathway (i.e. B to A). The two reactions are chemically distinct and as such, are catalysed by separate enzymes. The substrate cycle exists when the two enzymes are simultaneously catalytically active. For every turn of a substrate cycle (i.e. A to B to A), chemical energy is converted to heat, (usually from the cleavage of the high energy phosphate bonds of ATP to ADP, AMP, and inorganic phosphate) which is then used by the animal to maintain body temperature, or is lost to the environment (Newsholme and Crabtree 1976; Newsholme 1986).

Newsholme and Crabtree (1976) list several different substrate cycles, and discuss that the two possible roles of substrate cycling, HP and amplification, are intimately linked. Heat production is unavoidable as a result of cycling, but Newsholme (1976) noted that the amount of heat produced may set an upper limit to the amount of sensitivity that substrate cycling permits. Newsholme and Crabtree (1976) also said that opposing reactions in a substrate cycle were previously thought to never be simultaneously active because a waste of biological energy would result, possibly leading to fatal heat generation. They discuss the point that sensitivity can be maximal over a short period, without excessive accumulation of heat. However, long term effects of substrate cycling may be involved in weight control and

prevention of fat deposition, but measures to prevent heat accumulation would have to be taken. However, HP due to substrate cycling may not be limited by hyperthermia under cold climatic conditions, especially as this heat would be utilized to maintain body temperature due to increased heat loss to the environment. Substrate cycling has been discussed in relation to obesity in humans (Newsholme 1978; Astrup et al. 1989), but has also been involved in explaining the control of temperature regulation in domestic animals (Newsholme 1976; Reeds 1987) and humans (Wolfe et al. 1987).

Several authors have suggested that E and NE increase the rate of cycling between fructose-6-phosphate and fructose-1,6-bisphosphate in muscle during periods of anticipation or stress, and TAG and FFA in both BAT and white adipose tissue (WAT) (Newsholme 1976; Brooks et al. 1982, 1983; Challiss et al. 1984a, 1984b). These increased cycling rates are beneficial in preparing the animal for increased flux through the pathways during stress, as well as in converting chemical energy to heat. This mechanism is consistent with the anticipatory role of catecholamines and their established thermogenic action (Newsholme 1986; Newsholme et al. 1987).

Crabtree and his coworkers (1987) decided to investigate the rate of substrate cycling between acetate and acetyl-CoA in sheep muscle, in order to estimate the contribution of this cycle to total HP. Their results indicated that this cycle only contributes approximately 0.5% of the total HP in the animal. However, Jessop and his colleagues (1990) investigated this same cycle in sheep liver, and found that the HP may be as high as 2.5% of the basal HP. They concluded that this may be

partly responsible for the increased heat increment of feeding after ingestion of high acetate-providing forages. It would be of interest to measure the same cycle before and following acute and chronic cold exposure, as the use of fatty acid β-oxidation for HP increases during cold exposure.

The Cori cycle is an energetically wasteful process which involves the recycling of glucose from lactate by the liver. Lactic acid, produced by anaerobic glycolysis in many tissues, including muscle, is taken up by the liver and converted to glucose, which can be converted to glycogen or transported back to the glycolytic tissues to complete the cycle. This cycle is known to be stimulated by β_2-adrenergic agonists and is considered to be thermogenic because it requires two more moles of ATP than if glucose is directly converted to glycogen. This pathway may be a major contributor to thermogenesis because a significant amount of lactic acid is produced in WAT (Astrup et al. 1989).

One of the substrate cycles which appears to be greatly affected by β_2-adrenergic agonists is protein turnover. However, results are accumulating that indicate that β_2-agonists do not increase the rate of substrate cycling. β_2-agonists have been of interest lately because of their use as repartitioning agents which decrease the fat component and increase the muscling of carcasses (Stock and Rothwell 1986).

Newsholme et al. (1987) has cited the protein/amino acid cycle in his list of thermogenic substrate cycles. β_2-agonists, such as clenbuterol, have been shown to increase protein accretion in skeletal muscle (Reeds et al. 1986; Reeds 1987; Higgins et al. 1988), but some of

these studies have also shown that the effect of these β_2-agonists have been a reduction in protein degradation, rather than an increase in protein synthesis. This implies that the cycle is not accelerated, but instead is inhibited and therefore could possibly decrease HP. It has been suggested, however, that an increase in protein accretion in muscle and other organs, such as liver, account for the increase in resting metabolic rate seen with the administration of β_2-agonists. This increase in mass and volume of tissue increases the need for metabolic support (Astrup et al. 1989). β_2-agonists may act on the β_2-adrenoceptors found on skeletal muscle to cause protein accretion, by an as yet unknown mechanism.

β_2-agonists promote the transfer of calcium ions into the cytosol from the sarcoplasmic reticulum, which results in an increase in muscle tone due to activation of the contractile mechanism. The calcium pump within the membrane of the sarcoplasmic reticulum is an ATP-requiring enzyme which also results in HP from ATP hydrolysis (Astrup et al. 1989). Reeds (1987) has suggested that β_2-agonists, like clenbuterol and trenbolone, act at the level of the proteolytic enzymes. Higgins (et al. 1988) studied the activities of calpain proteinases and their inhibitor calpastatin in lambs treated with clenbuterol. She found that clenbuterol increased the activities of both the high calcium-requiring calpain II, which acts to break down myofibrillar proteins at the Z-lines, and calpastatin, which inhibits the action of the peptidases. However, calpain I activity did not increase, thus the calpastatin increase would decrease calpain I activity. This does not support the idea that protein turnover is increased by β_2-agonists and therefore

this process is not a thermogenic substrate cycle. Although there is plenty of research on substrate cycling, the lack of consensus as to their contribution to thermogenesis has led some authors to refute their significance (Harris and Lobley 1991; Rennie et al. 1992). Substrate cycles have been studied in several monogastric species, but to a small extent in ruminant species. The few studies performed in ruminants (Crabtree et al. 1987; Jessop et al. 1990) indicate that substrate cycles may not have a major contribution to whole body HP. It may be of interest to study substrate cycles in CA ruminants because in past experiments researchers showed enhancement of HP by high acetate-producing feedstuffs through the acetate/acetyl-CoA cycle at thermoneutral temperatures.

SODIUM-POTASSIUM PUMP AND CATECHOLAMINES

Milligan and McBride (1985) have shown that transport of sodium and potassium ions across the plasma membrane at rest, at the expense of ATP hydrolysis, accounts for approximately 20% of the energy expenditure in skeletal muscle, intestine and liver of ruminants. The energetic cost of maintaining this balance is increased upon exposure to cold, among other factors. The increased activity of these pumps is evident from an increase in oxygen uptake of hepatocytes and is inhibited by ouabain (Milligan and McBride 1985).

Milligan and McBride (1985) also proposed that since large adult mammals do not have detectable amounts of BAT, perhaps skeletal muscle contributes more substantially to thermogenesis than does BAT in

rodents. They gave evidence from results of chronically cold-exposed sheep whose skeletal muscle exhibited 50% higher oxygen uptake, compared to warm-acclimated sheep. The proposed mechanism of Na^+,K^+-ATPase activation by catecholamines is thought to be by increased permeability to cations following β-adrenoceptor stimulation, leading to an elevation of intracellular sodium concentration (Webster 1974a). It has also been found that catecholamines also increase Na^+,K^+-ATPase activity in rat soleus muscle via stimulation of β_2-adrenoceptors (Flatman and Clausen 1978; Clausen and Flatman 1980). Herpin and his colleagues (1987) have also stated that the increase in the activity of the Na^+,K^+-ATPase pump accounted for 70% of the increased oxygen consumption in the skeletal musculature of cold-exposed pigs. They also suggested this mechanism is important for cold-induced thermogenesis.

Increased energy expenditure at the Na^+,K^+-ATPase pump in skeletal muscle has been associated with higher growth rate of young calves and lambs. This pump is essential in allowing hypertrophic and hyperplastic growth to occur. Perhaps this mechanism is involved in thermogenesis during protein accretion induced by β_2-agonist administration (Milligan and McBride 1985).

OBJECTIVES

Thermogenesis has been shown to be influenced by several mechanisms, some of which are thought to be induced by catecholamines. It is most likely that several or all of these mechanisms act in concert during cold exposure, since catecholamines affect almost every tissue in

multiple ways upon their release. Brown adipose tissue mitochondria use thermogenin to alter heat production by decreasing the coupling of electron transport and oxidative phosphorylation. It is not clear that any other thermogenic tissues use similar mechanisms to produce heat since no comparable protein has yet been found in other tissues. Herpin (1988) has shown that rhomboideus muscle in cold-acclimated piglets may have a coupling defect in the subsarcolemmal mitochondria. This suggests the existence of NST in this muscle.

Other mechanisms exist for thermogenesis which can be grouped together as substrate cycles. Substrate cycling to produce heat involves acceleration of opposing reactions in a metabolic pathway such that there is net ATP hydrolysis to produce heat. Examples of substrate cycles include lipolysis-lipogenesis, protein turnover, the Cori cycle, and ionic pumps. The Na^+,K^+-ATPase pump appears to be the most feasible and the most obvious thermogenic mechanism, as it is ubiquitous in body tissues, and its function for homeostasis accounts for a relatively large percentage of total energy expenditure.

Several gaps in information exist with respect to how or if catecholamines may contribute to cold-induced increase in HP. It is not known how much of the increase in HP is attributable to catecholamine stimulation, even though it is known that catecholamine levels are increased in cold-exposed animals. Very little information is available as to the normal plasma metabolite levels in cold exposed ruminants, especially those associated with supplying energy substrates for HP, and those that indicate certain tissues are being mobilized for HP. These include (1) glycerol, as an indication of lipolysis, (2) plasma urea

nitrogen, as an indication of amino acid metabolism in the liver for gluconeogenesis, and (3) plasma amino acid levels, which, if elevated, may imply general protein mobilization. The effects of E infusion on parameters such as HP, cardiac output, glucose and FFA has been studied, but not many researchers have looked at glycerol, PUN, or plasma amino acid levels following catecholamine infusion. Another problem yet to be addressed is whether the dosages used actually produce plasma catecholamine concentrations which are within physiological ranges, and thus make the results of such studies more credible.

The objectives of this study are to determine whether HP is greater, and whether plasma catecholamine levels are higher in CA versus WA sheep. The first experiment will reveal if levels of HP and plasma catecholamines and metabolites used for HP are higher in CA versus WA sheep. Further, whether the use of β_1- versus β_2-blockers can differentially change the concentrations of energy substrates measured in plasma and decrease the HP in CA animals. The results would reveal if catecholamines, acting on β-receptors, are responsible for a portion of the cold-induced change in HP, and if β_1- and β_2-receptor subtypes have differential effects on metabolic responses during cold exposure.

In the second experiment, 2 CA and 2 WA sheep from the first experiment will be infused with a single level of each β-adrenergic antagonist, with and without E infusion. The same parameters, HP and plasma metabolites, as well as plasma catecholamines, will be measured with 2 objectives in mind. First, it is of interest to see if either β_1- or β_2-adrenergic antagonist could change any E-induced change in HP

or plasma metabolite concentration. Second, a difference in response of CA and WA animals to E infusion could be elucidated.

MATERIALS AND METHODS

EXPERIMENT ONE

Husbandry and Experimental Design. Yearling Suffolk wethers were acclimated for 3 wk to either 26°C (WA = warm acclimated), or 2°C (CA = cold acclimated). The animals were initially shorn to a fleece depth of 0.5 cm. The fleece depth was not allowed to exceed 1 cm by shearing at 2 wk-intervals during the experiment.

All the animals were selected for similarity in body weight, the average weight being 43.3 ± 1.21 kg. The animals were weighed every 2 wk, as well as before and immediately following each infusion sequence.

There were six animals in each group (ie. WA and CA groups), for a total of 12 animals. The animals were divided into pairs, with each pair denoted as WA1, CA2, and so on. Each pair was given seven treatment infusions (Table 1) over seven to eight consecutive days, with each 3-h infusion given on a single day between 09:00 and 15:00 h. The order of the infusions was varied to produce three different sequences. WA1 and CA2 received the same infusion order, WA3 and CA4 received the same order, and so on. The order of infusions was such that each animal did not receive the same β-receptor subtype antagonist, or a high dose of antagonist, two days in a row. The dose rates for both antagonists were 2.5, 5.0, or 10.0 $\mu g\ kg^{-1}\ min^{-1}$. These dosages respectively

corresponded to the designations of B1L, B1M, and B1H for the β_1-antagonist, and to B2L, B2M, and B2H for the β_2-antagonist.

Room lighting consisted of a light photoperiod from 06:00 h to 22:00 h. The sheep were fed once daily at 16:00 h, at least one hour following the daily treatment infusion. The diet consisted of alfalfa hay (midbloom, ME = 8.8 MJ kg^{-1}, 12% crude protein). Warm-acclimated and CA animals were respectively fed 1.2 and 1.5 kg of hay per day. Water and cobalt-iodized salt were offered ad libitum. All sheep were housed in individual metabolism crates. The CA animals were housed as pairs, in each cold room, whereas all six of the WA animals were housed in one room.

Each animal was implanted with two bilateral polyvinyl chloride jugular cannulae (PVC 105-18, Alpha Wire Corp.) at least 1 d prior to the infusion period. The cannulae were inserted into the jugular vein 10 cm from the site of cannulation. The cannulae were long enough (about 4 m) so that they could be extended out of the experimental room through portholes such that blood samples could be obtained, and the infusate could be administered, without disturbing the animals. The dead space of each cannula was approximately 7 mL. The right cannula was used for blood sampling, and the left cannula was used for infusing solutions. These cannulae were stored nightly around the animal's neck in the pockets of a home-made elastic Velcro^R neck band.

At the beginning of the day, the feed and water bins were removed and replaced with metabolic hoods, for the measurement of whole body oxygen consumption. Once the animal's head was secured in the hood, the jugular cannulae were extended through portholes, and the rectal

temperature was taken. The rectal temperature of each animal was taken before and after the daily infusion, as a means of monitoring health status. All jugular cannulae were checked for patency using heparinized physiological sterile saline. Each animal was maintained in a standing position by securing binder twine below its abdomen, which discouraged the animal from lying down during the infusion. The twine was removed at the end of each infusion.

While the animal and gas level were allowed to equilibrate, the oxygen analyzer and infusion pump (Pharmacia Peristaltic Pump P3, Pharmacia Fine Chemicals, Uppsala, Sweden) were calibrated, and the infusion solution was prepared.

Once the animal had reached a relatively constant level of oxygen consumption (after at least an hour), a permanent record was obtained and used as the -0:30 h preinfusion measurement and a 10 mL blood sample was taken. There was approximately 14 h between the last observed feed consumption and the first blood sampling in the morning. At time 0:00, another blood sample was obtained, then a primer bolus of the antagonist was given (if applicable), and then the cannula was flushed with sterile saline. The cannula was attached to the infusion pump, and the infusion began. Subsequently, oxygen consumption was recorded at 15-min intervals, and 10 mL blood samples were obtained at 1:00 h, 2:00 h, and 3:00 h during the infusion.

Infusion Solutions. The β_1-adrenergic antagonist was metoprolol tartrate (Sigma Chemical Co., St. Louis, MO), and the β_2-antagonist was ICI 118,551 (donated by ICI Pharmaceuticals, Wilmington, DE, and

Macclesfield, England). The daily infusate was made up in isotonic sterile saline solution (0.9% NaCl) in sterilized brown bottles, and aluminum foil was used to prevent excessive light exposure. Ascorbic acid (0.1 g L^{-1}) was added as an antioxidant. Because of the limited amount of β_2-antagonist available, the animals in the same pair were given the same infusion on the same day. A single pair of animals were infused per day. The infusion rate was 0.5 mL min^{-1} over a total time of 180 min, therefore, a total of 200 mL of infusate was prepared. Primer injections (1 mg kg^{-1}, in a 3 mL total volume) of the β-antagonists were given just prior to the beginning of the infusion.

Heat Production. Heat production was estimated from oxygen consumption determined in an open-circuit respiratory apparatus (Young et al., 1975). Respiratory gas was collected using ventilated hoods, to which the animals had been previously accustomed. The hoods were ventilated continuously and a flowmeter (Rotameter, Fisher and Porter, Warminster, PA) was used to measure the ventilation rate of the hood. A dual-channelled paramagnetic oxygen analyzer (Servomex OA184, Sussex, England) was used to measure the difference in oxygen concentration between the air entering the hood and the air leaving the hood. This difference, multiplied by ventilation rate (STPD), was used as an estimate of the oxygen consumption by the animal. Nitrogen gas was used to calibrate the oxygen analyzer to zero, and the gravimetric iron-burner method was used for whole-system calibration (Young et al. 1984). Heat production (kJ $kg^{-0.75}$ min^{-1}) was calculated using the equation formulated by McLean (1972).

Blood Sampling. Blood was sampled (10 mL) at -0:30 h, 1:00 h, 2:00 h, and 3:00 h during the infusion. The sample was mixed with 200 uL of a 5 mM EDTA solution, and placed on ice for a maximum of 15 min before it was centrifuged for 15 min at 1600 g in a 4°C centrifuge (Beckman J6M/AE, Palo Alto, CA). A single plasma sample was divided into five portions in microcentrifuge tubes, and quickly frozen in liquid nitrogen. The portion for catecholamine analysis was stored in liquid nitrogen prior to analysis. The other portions were stored at -40°C until analyses for glycerol, glucose, PUN, and amino acids could be performed.

Plasma Analyses. Plasma glycerol was determined using a colorimetric test kit (Boehringer Mannheim, Laval, PQ) and a spectrophotometer (Milton Roy Spectronic 3000 Array, USA). The samples were first deproteinized with 0.125 M zinc sulfate and 0.250 M sodium hydroxide, as described by Wieland (1984). The principle of the determination involves the phosphorylation of glycerol by adenosine-5'-triphosphate (ATP) to glycerol-3-phosphate, which is catalyzed by glycerokinase. The adenosine-5'-diphosphate (ADP) formed in this reaction is reconverted into ATP, with the concurrent formation of pyruvate, by phospho-enolpyruvate and pyruvate kinase. The resulting pyruvate is reduced to L-lactate by reduced nicotinamide-adenine dinucleotide (NADH), in the presence of lactate dehydrogenase. NADH is oxidized to NAD in this reaction, and is stoichiometric with the amount of glycerol in the sample. The amount of NADH is determined spectrophotometrically at 340 nm.

Glucose in plasma was determined using a quantitative, enzymatic test kit (Sigma Diagnostics, St. Louis, MO), following the procedure of Trinder (1969) and a spectrophotometer (Milton Roy Spectronic 3000 Array, USA). The glucose in the sample is first oxidized to gluconic acid and hydrogen peroxide, in the reaction catalyzed by glucose oxidase. Hydrogen peroxide then reacts in the presence of peroxidase with 4-aminoantipyrine and p-hydroxybenzene sulfonate to form a quinoneimine dye, with an absorbance maximum at 505 nm. The glucose concentration in the sample is directly proportional to the intensity of the color produced (Trinder 1969).

Plasma urea nitrogen was determined using a quantitative colorimetric test kit (Sigma Diagnostics, St. Louis, MO) and a spectrophotometer (Milton Roy Spectronic 3000 Array, USA). The principle of the determination is based on the procedure of Crocker (1967), with deproteinization of the sample using cold 3 % trichloroacetic acid. Diacetyl monoxime reacts with urea to form hydroxylamine and a pink chromogen. The urea concentration is directly proportional to the intensity of the color produced, which is measured spectrophotometrically at 525 nm (Crocker 1967).

Plasma amino acids were derivatized using the o-phthaldialdehyde method (Jones and Gilligan, 1983). Amino acids were quantified using a Varian 5000 high performance liquid chromatograph and a Varian Fluorichrom detector (Toronto, ON). Prior to injection, the samples were mixed 1:1 with a fluoraldehyde reagent, with a delay time of 12 sec. A modified Technicon autosampler (Montreal, PQ) and a Chemlab peristaltic pump (Mississauga, ON) with a stainless steel mixing tee

were used to mix the samples. Samples were injected using a Valco autoinjector valve equipped with a 20 μL loop. A Supelcosil 3 micron LC-18 reverse phase column (4.6 x 150 mm; Supelco, Oakville, ON) was used to separate the amino acids, equipped with a guard column (4.6 x 50 mm) packed with Supelco LC-18 reverse phase packing (20-40 μm). The chromatographic peaks were recorded using a Fisher Recordall recorder, and integrated using a Hewlett Packard 2645 data system with a Hewlett Packard 18652A A/D converter (Mississauga, ON).

Determination of plasma catecholamines was performed using HPLC, following the procedure of Kilpatrick (et al. 1986) and Bioanalytical Systems Inc. (LCEC Application Note No. 14). The samples were injected using a CMA200 refrigerated microsampler (BAS, West Lafayette, IN) and a dual-pistoned Waters 590 pump (Millipore, Missisaugua, ON). An Econosil C18 5 μm particle column (4.6 x 250 mm, Alltech/Applied Science) was used to separate the catecholamines. Chromatographic peaks were detected using an ESA Coulochem Model 5100A Coulometric Detector (Scientific Products and Equipment, Concord, ON). Integration was accomplished using a SP4290 Spectra-Physics Integrator (Alar Science Inc., St. Albert, AB) and a Packard Bell PB8810 computer.

For the glycerol, glucose, urea nitrogen, and plasma amino acids, the -0:30 h and 3:00 h samples were analyzed. For the plasma catecholamines, only the -0:30 h samples were analyzed.

Statistical Analyses. The data was analyzed by least square analysis of variance (ANOVA) using the SAS general linear model (GLM) procedure (SAS Institute 1989). The model included the temperature, sequence of

infusions, animal, dose, and the various interactions of these factors. The pre- to postinfusion difference least square treatment means (LS means) were tested for significance, compared to saline infusion, using the Student's t-test (Steel and Torrie 1980). Dose response curves were calculated using multiple regression.

Table 1: Order of infusions for each warm- (WA) and cold-acclimated (CA) pair of sheep, where β_1 - metoprolol, and β_2 - ICI 118,551 ($\beta_{1,2}$ low - 2.5 $\mu g\ kg^{-1}\ min^{-1}$; $\beta_{1,2}$ medium - 5.0 $\mu g\ kg^{-1}\ min^{-1}$; $\beta_{1,2}$ high - 10.0 $\mu g\ kg^{-1}\ min^{-1}$) in Experiment One.

	Pairs of Animals		
Infusion	WA1 and CA2	WA3 and CA4	WA5 and CA6
1	saline	β_2 low	β_1 low
2	β_1 low	β_1 high	β_2 medium
3	β_2 high	β_2 medium	β_1 high
4	β_1 medium	β_1 medium	saline
5	β_2 medium	β_2 high	β_2 high
6	β_1 high	β_1 low	β_1 medium
7	β_2 low	saline	β_2 low

EXPERIMENT TWO

Four sheep (WA5 and CA6) from Experiment One, were fed and managed as outlined previously, and given six treatment infusions over six to seven consecutive days. This experiment was conducted within a week of the completion of Experiment One. On a single day, the animals received either (1) sterile physiological saline (with 0.1 g L^{-1} ascorbic acid), (2) a β_1-antagonist (metoprolol, 5.0 μg kg^{-1} min^{-1}), (3) a β_2-antagonist (ICI 118,551, 5.0 μg kg^{-1} min^{-1}), (4) epinephrine (0.3 μg kg^{-1} min^{-1}), (5) epinephrine (0.3 μg kg^{-1} min^{-1}) plus metoprolol (5.0 μg kg^{-1} min^{-1}), or (6) epinephrine (0.3 μg kg^{-1} min^{-1}) plus ICI 118,551 (5.0 μg kg^{-1} min^{-1}), between 09:00 and 15:00 h. These treatments were designated as S, B1, B2, E, EB1, and EB2, respectively. Epinephrine hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO). All solutions contained 0.1 g L^{-1} ascorbic acid and were prepared as outlined in Experiment One.

While the WA sheep were infused in their acclimation room, the CA sheep were moved, at least an hour prior to the start of the experiment, from their 2°C room, to the 26°C warm-acclimation room. These animals had been previously accustomed to the daily move, and were replaced into the cold chamber after the completion of the daily experiment. Primer injections of the antagonists were given as in Experiment One. Heat production, blood sampling, and plasma analyses were all measured and performed as described for Experiment One. For the plasma catecholamines, both the -0:30 h and the 3:00 h samples were analyzed, and the difference between these two samples was calculated. Data were

analyzed as a change from preinfusion. Significant LS means were tested using the Student's t-test.

RESULTS

EXPERIMENT ONE

Effects of Infusion Sequence. From the statistical analysis of the data (simple comparison of means using a Student's t-test), it was determined that there were no significant effects of the infusion order on any of the parameters measured ($P>0.05$).

Effects of Temperature. The preinfusion rectal temperatures are presented in Table 2. The CA animals average rectal temperature was 37.4°C. This was a significant decrease ($P<0.0007$) of 1.0°C from the WA animals (38.4°C).

The preinfusion HP in the CA animals was 131% higher ($P=0.0001$) than that of the WA animals (Table 2). The CA animals had an average preinfusion HP of 0.486 kJ $kg^{-0.75}$ min^{-1}, while the WA animals' average preinfusion HP was 0.210 kJ $kg^{-0.75}$ min^{-1}.

Plasma glycerol was significantly higher ($P<0.002$) in the CA animals (0.432 mg dL^{-1}), as compared to the WA animals (0.213 mg dL^{-1}). Plasma glucose concentration of 60.56 mg dL^{-1} in the CA animals was 17% higher ($P=0.0007$) than that (51.88 mg dL^{-1}) in the WA animals. Plasma urea nitrogen levels, were significantly lower ($P<0.04$) in the WA animals (16.66 mg dL^{-1}), compared to the CA animals (23.25 mg dL^{-1}).

With respect to the free amino acids measured in the plasma samples (Table 3), valine, isoleucine, leucine and total amino acids were greater in the CA animals (333, 128, 189 and 2801 nmol mL^{-1}, respectively) compared to the WA animals (177, 96, 108 and 2640 nmol mL^{-1}, respectively). However only the valine, isoleucine, and leucine levels were significantly different between the two temperatures ($P<0.003$). By contrast, the glutamine and alanine levels were lower in the CA animals (258, 121 nmol mL^{-1}), compared to the WA group (343, 148 nmol mL^{-1}), however only the glutamine levels were significantly lower ($P<0.009$).

The preinfusion E and NE least square means for the WA and CA groups are shown in Table 4. The E concentrations in the CA animals were more than twice that of the WA animals, however, the difference between the two groups only approached significance. The preinfusion NE levels were significantly greater ($P<0.04$) in the CA group, as compared to the WA group.

Effects of β-Adrenergic Antagonist Infusion.

Heat Production-The WA animals had no significant changes in HP following any of the metoprolol infusions (Figure 1). In the CA animals (Figure 1), the decreases in HP during infusion of 2.5 (B1L) and 10.0 (B1H) $\mu g\ kg^{-1}\ min^{-1}$ metoprolol were slightly, but not significantly greater than the decrease in HP during saline infusion. The difference in HP between the saline and 5.0 (B1M) $\mu g\ kg^{-1}\ min^{-1}$ metoprolol infusions only approached significance ($P<0.09$). The reduction in HP during B1M

infusion was equivalent to 15% of the additional preinfusion HP in the cold compared to the WA animals.

There were no significant differences between the reductions in HP during any of the β_2-antagonist infusions versus the saline infusion in the WA animals (Figure 2). In the CA animals (Figure 2), both the 2.5 (B2L) and 5.0 (B2M) $\mu g\ kg^{-1}\ min^{-1}$ doses of ICI 118,551 caused decreases in HP that approached significance ($P<0.09$) when compared to the saline infusion results. These two reductions in HP represented changes of 13 to 14% of the additional preinfusion HP in the CA compared to the WA animals.

Glycerol-In the WA animals, there were no significant effects of infusing any of the three levels of either metoprolol (Figure 3) or ICI 118,551 (Figure 4), on the levels of glycerol in the plasma, as compared to the effects of saline infusion. However, there were multiple significant effects observed in the CA animals. The low level of metoprolol (Figure 3) significantly ($P<0.04$) decreased plasma glycerol by approximately 52% of the preinfusion difference between the WA and CA animals, as compared to the effects of saline infusion. However, the medium and high levels of metoprolol did not affect plasma glycerol significantly, when compared to the effects of saline infusion. All three levels of ICI 118,551 (Figure 4), B2L, B2M, and B2H, significantly decreased plasma glycerol levels ($P<0.04$, 0.002, and 0.0001, respectively), when compared to the effects of saline infusion. The reduction of plasma glycerol increased in direct proportion to the dose of ICI 118,551. These effects represent approximately 14%, 49% and 93%

reductions from preinfusion differences between the WA and CA animals, for the B2L, B2M, and B2H infusions, respectively.

Glucose-Even though the CA animals had higher plasma glucose levels overall when compared to the WA animals, there were no significant effects of infusing either the β_1- or the β_2-antagonists, compared to saline infusion (Figures 5 and 6), in either the WA or CA animals.

Plasma Urea Nitrogen-The preinfusion PUN levels were significantly higher in the CA animals, as compared to the WA animals. However, neither metoprolol or ICI 118,551, at any of the three dose levels, had significant effects on PUN, when compared to the effects of saline infusion, in either WA or CA animals (Figures 7 and 8).

Amino Acids-Although there was a trend for the difference in total amino acids to increase with increasing dose of metoprolol, in both the WA and CA groups, none of these changes were significantly different compared to the saline infusion (Figure 9). The responses to different doses of ICI 118,551 in the WA animals were not significantly different from saline infusion (Figure 10). In the CA animals, the B2M infusion increased the total plasma amino acid concentration compared to saline infusion. The increase was approximately 55% of the preinfusion level, and approached significance ($P<0.06$). The values obtained following the B2L and B2H infusions were not significantly different from saline infusion (Figure 10).

There were no significant effects of metoprolol infusion on either groups' average plasma alanine concentration, when compared to saline infusion (Figure 11). ICI 118,551 had no significant effect on plasma alanine levels, in either WA or CA animals (Figure 12).

In the WA group, only the B1M infusion significantly ($P<0.02$) increased the plasma glutamine concentration compared to saline. This change was approximately 73% of the preinfusion concentration. In the CA group, there was a trend towards increasing glutamine levels with increasing metoprolol dose, however none of these effects were significantly different from saline infusion (Figure 13). ICI 118,551 had no significant effects on the glutamine levels in the WA group, however the B2M infusion significantly ($P<0.04$) increased the glutamine level in the CA group, with the increase representing approximately 70% of the preinfusion concentration (Figure 14).

Metoprolol did not have any significant effects on either the WA or CA animals' plasma valine levels (Figure 15). ICI 118,551 did not significantly affect the WA animals, however, in the CA group, the B2M infusion significantly ($P<0.03$) increased the valine level compared to saline infusion (Figure 16). The increase in valine was about 37% of the preinfusion value.

In the WA animals, plasma leucine levels were not significantly affected by the infusions of metoprolol and ICI 118,551 (Figures 17 and 18). Likewise, metoprolol did not significantly affect the leucine levels in the CA animals, when compared to saline infusion (Figure 17). However, the B2M infusion significantly ($P<0.02$) increased the plasma leucine level compared to the effects of saline infusion in the CA group

(Figure 18). The change from preinfusion was approximately a 48% increase.

Plasma isoleucine levels in both the WA and CA groups were not significantly affected by the metoprolol infusions (Figure 19). Similarly, ICI 118,551 did not significantly affect isoleucine levels in the WA animals, when compared to saline. However, the B2M infusion significantly ($P<0.03$) increased isoleucine levels in the CA animals (Figure 20), with the change representing approximately 40% of the preinfusion level.

Dose Response Curves-The results of the significant ($P<0.05$) multiple regression analyses are shown in Figures 21 to 25. The corresponding curves for WA or CA animals are also shown for comparison, even if they were not significant.

The response of HP to increasing doses of ICI 118,551 fit a quadratic regression (Figure 21). The equation for the WA animals was significant ($P<0.04$), however, that for the CA animals only approached significance. The WA group showed the greatest decrease in HP at the B2H dose, and the smallest decrease at the B2M dose. By contrast, the CA group showed the greatest decrease in HP at the B2M dose, and the smallest decrease at the zero dose.

Figure 22 shows the cubic regressions for the responses of plasma glycerol to increasing metoprolol dose. For both the WA and CA groups, the largest increase in plasma glycerol occurred at the B1M dose, and the largest decreases in plasma glycerol occurred at the B1L and B1H doses. The CA animals' response to metoprolol fluctuated more widely

than the WA animals, as is shown by the greater amplitude of the curve's peak and valley. The cubic regression was significant ($P<0.04$) for the CA group, but not for the WA group.

There was a linear decrease in plasma glycerol in response to increasing doses of ICI 118,551 (Figure 23), in the CA animals, whereas the regression was not significant in the WA animals.

The response of plasma glutamine level to increasing metoprolol dose was described by a quadratic relationship (Figure 24) in WA animals ($P<0.02$). The WA animals showed the greatest increase in plasma glutamine levels at the B1M dose, with smaller increases at the B1L and B1H doses. The CA animals' glutamine levels tended to increase with increasing metoprolol dose, but the regression was not significant; nor was there a significant linear effect of dose in the CA animals.

The responses of plasma glutamine to increasing doses of ICI 118,551 are shown in Figure 25. Quadratic relationships best described the data from the WA and CA groups, however, only the CA animals' regression equation was statistically significant. The WA group showed the greatest decrease in glutamine at the B2M dose, and an increase at the B2H dose. Conversely, the CA group showed the greatest increase in glutamine at the B2M dose, with smaller increases at the B2L and B2H doses.

EXPERIMENT TWO

Effects of Temperature. The LS mean values for the measured preinfusion parameters for the WA and CA animals were similar to those in Experiment

One (Table 5). However there were only significant differences between WA and CA animals for the HP and valine concentrations, with the CA animals having higher values than the WA animals.

Effects of Epinephrine and β-Adrenergic Antagonist Infusion. There were two animals in each temperature group, however only one CA animal provided measurable levels of E and NE, for all the infusions. The preinfusion catecholamine sample from the same CA animal during the E infusion trial was lost. In order to calculate a change in E and NE concentrations during the E infusion for the CA animal, the average of the preinfusion concentrations for the other infusion treatments was used (2.83 ± 0 105 pmol mL^{-1} for E and 5.98 ± 0.945 pmol mL^{-1} for NE).

The absolute pre- and postinfusion E and NE concentrations are presented in Appendix Figures 1 and 2. The pre- to postinfusion differences in E and NE concentrations calculated from the absolute values are presented in Figures 36 and 37.

Saline versus Epinephrine-Infusion of E significantly changed several parameters when compared with the effects of saline infusion. Heat production, plasma glycerol, and plasma glucose concentrations were increased ($P<0.05$) during E infusion in both the WA and CA groups, however, the HP changes in the CA group only approached significance ($P<0.09$) (Figures 26). Plasma alanine (Figure 31) and E (Figure 36) concentrations in the WA animals increased ($P<0.05$) during E infusion, compared to during saline infusion. A statistical comparison of the CA animals' E concentration changes during saline and E infusions was not

possible, however, the change in E between the two infusions was numerically different (Figure 36). Plasma E concentration was increased almost six-fold in the WA and almost 13-fold in the CA animals following E infusion (Table 5 and Figure 36). There was no significant effect of E infusion on plasma NE concentrations, in either the WA or CA groups (Figure 37).

Saline versus Epinephrine plus β_1-Antagonist-The increases in plasma glycerol, glucose, and E concentrations were significantly different between the saline and the metoprolol plus epinephrine (EB1) infusions, in both the WA and CA animals (Figures 27, 28, and 36). Plasma urea nitrogen decreased significantly during EB1 infusion in the CA animals, compared to saline (Figure 29).

Saline versus Epinephrine plus β_2-Antagonist-Plasma glucose and isoleucine concentrations in both animal groups, and plasma leucine levels in the CA group, were significantly different, when comparing the ICI 118,551 plus epinephrine (EB2) and saline infusions (Figures 28, 34, and 35). The concentrations of these parameters were greater following the EB2 infusion.

Epinephrine versus Epinephrine plus β_1-Antagonist-Both the WA and CA groups showed significant changes in plasma glycerol concentrations when the E and EB1 infusions were compared (Figure 27). The EB1 infusion caused a significant decrease in plasma glycerol concentration, when compared to E infusion.

Epinephrine versus Epinephrine plus β_2-Antagonist-There were several significant changes observed between the EB2 and E infusions. Heat production in the WA animals, plasma glycerol in both groups, and glucose in the CA animals, were significantly lower following EB2 infusion (Figures 26, 27, and 28). Concentrations of PUN in the WA animals, plasma leucine and isoleucine in both groups, and E in the WA animals, were significantly increased following the EB2 infusion compared to the E infusion (Figures 29, 34, 35, and 36).

Epinephrine plus β_1-Antagonist versus Epinephrine plus β_2-Antagonist-When comparing the EB1 infusion changes to the EB2 infusion changes, it was observed that plasma glycerol concentrations in both animal groups were greater following EB1 infusion (Figure 27). Concentrations of PUN in the CA animals, and isoleucine in the WA animals were significantly lower following the EB1 infusion, compared to the EB2 infusion (Figures 29 and 35). Epinephrine concentrations in the WA animals were lower following EB1 treatment, compared to EB2 treatment. Conversely, E concentrations were greater in the CA animals following EB1 treatment, when compared to the EB2 treatment (Figure 36).

Table 2: The effects of different temperatures of acclimation (warm = WA = 26°C; cold = CA = 2°C) on preinfusion rectal temperature, heat production (HP), and plasma parameters in Experiment One (n = 42 unless otherwise noted by a superscript).

Parameter	WA animals	CA animals	SEM	Signif.
Rectal Temperature (°C)	38.4[a]	37.4[b]	0.08	0.0007
HP (kJ $kg^{-0.75}$ min^{-1})	0.210	0.486	0.0040	0.0001
Glycerol (mg dL^{-1})	0.213	0.432	0.0144	0.002
Glucose (mg dL^{-1})	51.88	60.56	0.555	0.0007
Urea Nitrogen (mg dL^{-1})	16.66	23.25	0.240	0.04

[a] n = 41

[b] n = 40

Table 3: The effects of different temperatures of acclimation (warm - WA = 26°C; cold - CA = 2°C) on preinfusion plasma amino acid levels (nmol mL^{-1} plasma), in Experiment One.

Parameter	WA animals (n = 40)	CA animals (n = 42)	SEM	Signif.
Total Amino Acids	2640	2801	81.5	0.50
Valine	177	333	9.0	0.002
Isoleucine	96	128	3.1	0.002
Leucine	108	189	5.7	0.003
Glutamine	343	258	11.2	0.009
Alanine	148	121	3.9	0.08

Table 4: The effects of different temperatures of acclimation (warm - WA - 26°C; cold - CA - 2°C) on preinfusion plasma epinephrine and norepinephrine levels (pmol mL^{-1}), in Experiment One (n - 42).

Parameter	WA animals	CA animals	SEM	Signif.
Epinephrine	0.749	1.500	0.0514	0.10
Norepinephrine	3.252	14.220	0.4456	0.04

Table 5: The effects of different temperatures of acclimation (warm - WA = 26°C; cold - CA = 2°C) on preinfusion heat production (HP) and plasma parameters at 26°C (AA - amino acids), in Experiment Two (n - 12 unless otherwise noted by a superscript).

Parameter	WA animals (WA5)	CA animals (CA6)	SEM	Signif.
HP (kJ $kg^{-0.75}$ min^{-1})	0.273	0.370	0.0088	0.02
Glycerol (mg dL^{-1})	0.357	0.303[a]	0.0201	0.61
Glucose (mg dL^{-1})	52.75	55.57[a]	0.942	0.60
Urea Nitrogen (mg dL^{-1})	16.83	17.01[a]	0.287	0.94
Total AA (nmol mL^{-1})	2540	2491[a]	81.5	0.92
Valine (nmol mL^{-1})	174	282[a]	8.6	0.05
Isoleucine (nmol mL^{-1})	87	109[a]	3.4	0.29
Leucine (nmol mL^{-1})	101	161[a]	5.5	0.11
Glutamine (nmol mL^{-1})	268	222[a]	9.1	0.40
Alanine (nmol mL^{-1})	146	160[a]	5.0	0.71
Epinephrine (pmol mL^{-1})	1.158	2.828[b]	0.1204	0.29
Norepinephrine (pmol mL^{-1})	4.027	5.981[b]	0.4749	0.15

[a] n = 11

[b] n = 5

Figure 1: The effects of various 3–h infusions of metoprolol (B1) on heat production in warm– and cold–acclimated wethers (* P<0.09)

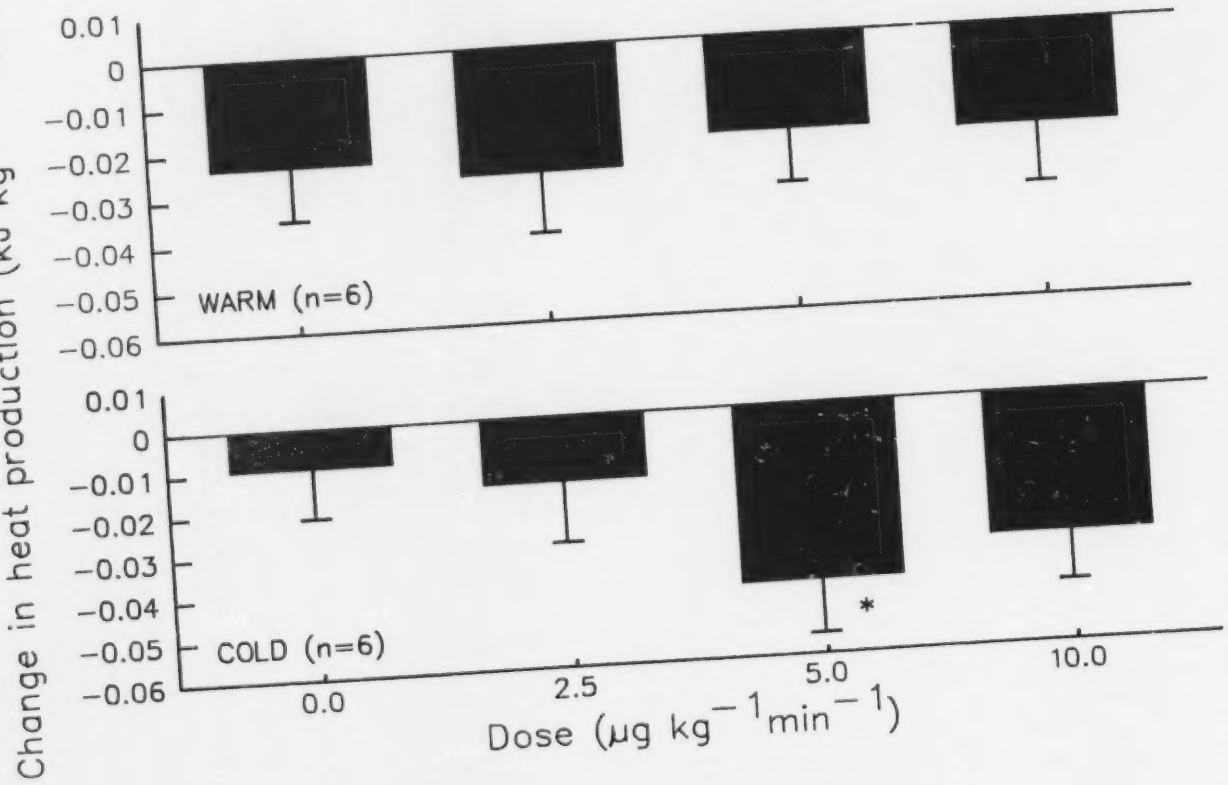


Figure 2: The effects of various 3–h infusions of ICI 118,551 (B2) on heat production in warm– and cold–acclimated wethers (* P<0.09)

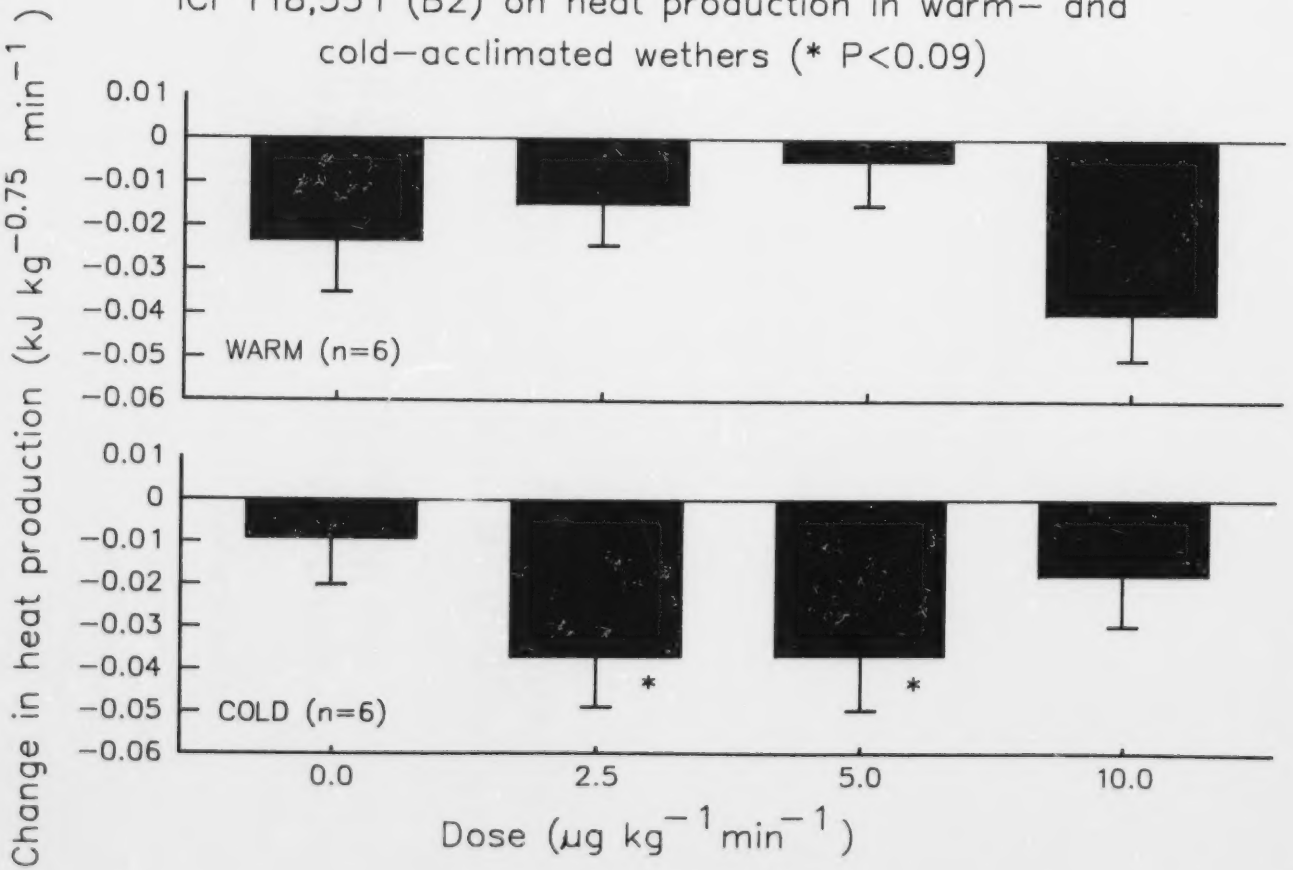


Figure 3: The effects of various 3–h infusions of metoprolol (B1) on plasma glycerol in warm– and cold–acclimated wethers (* P<0.04)

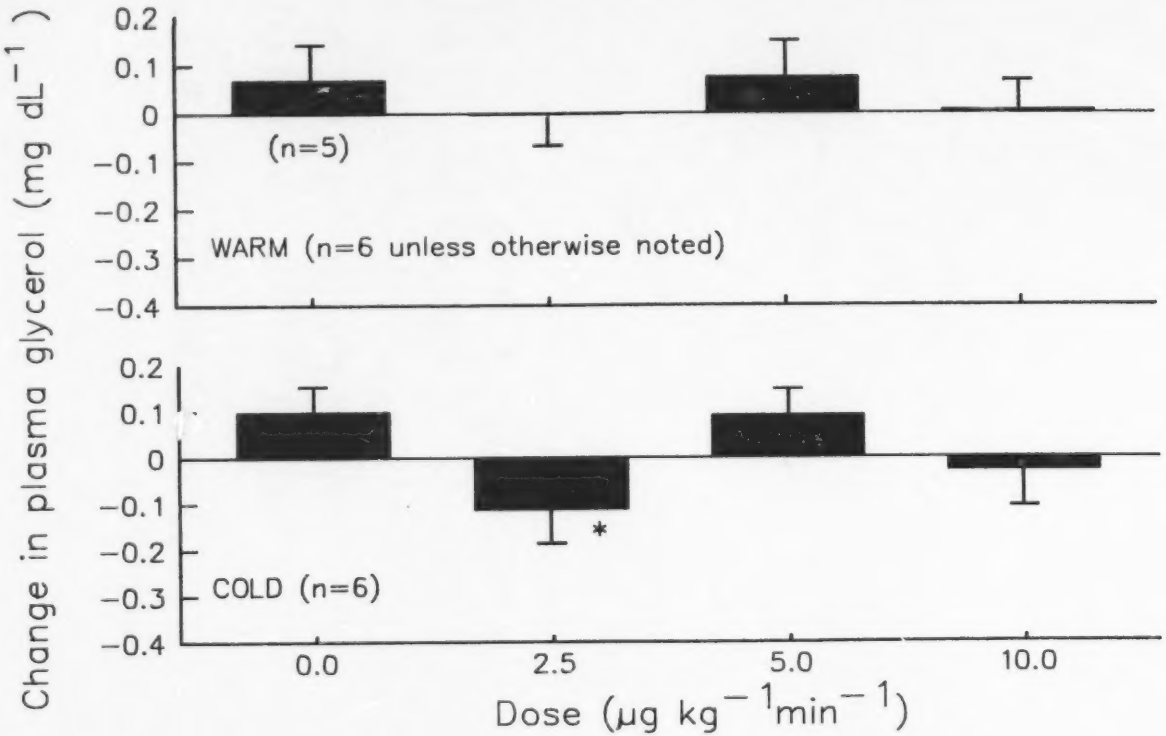


Figure 4: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma glycerol in warm– and cold–acclimated wethers (* P<0.04;** P<0.003;*** P<0.0001)

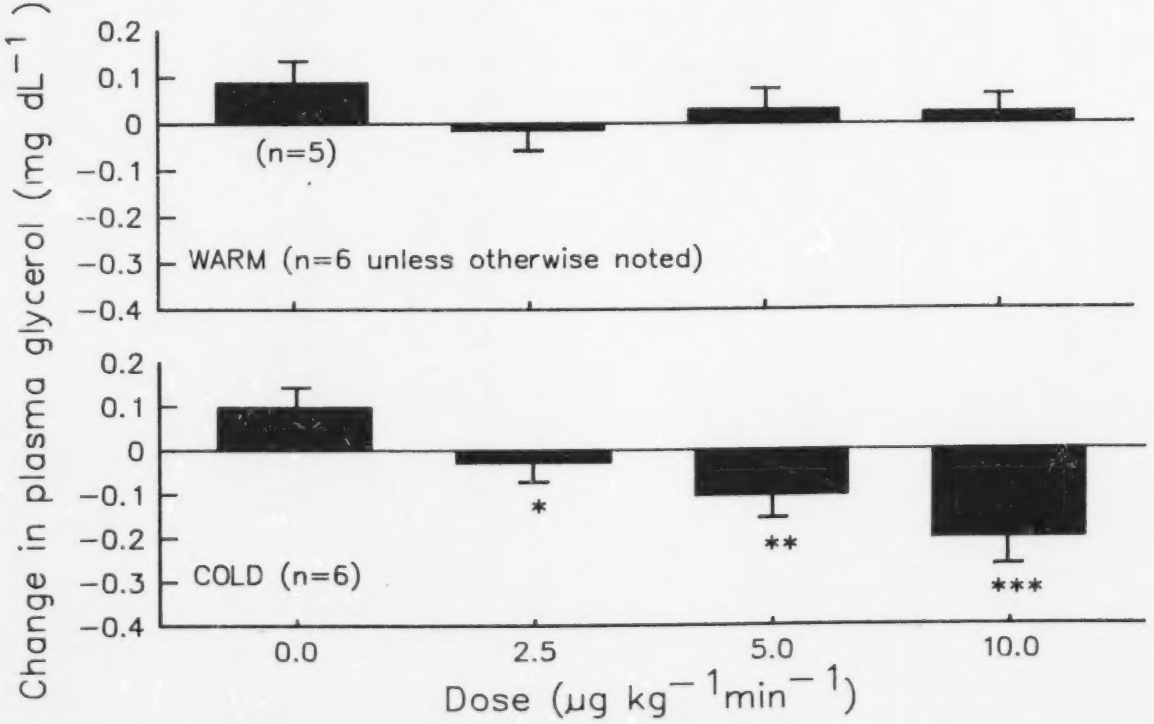


Figure 5: The effects of various 3–h infusions of metoprolol (B 1) on plasma glucose in warm– and cold–acclimated wethers

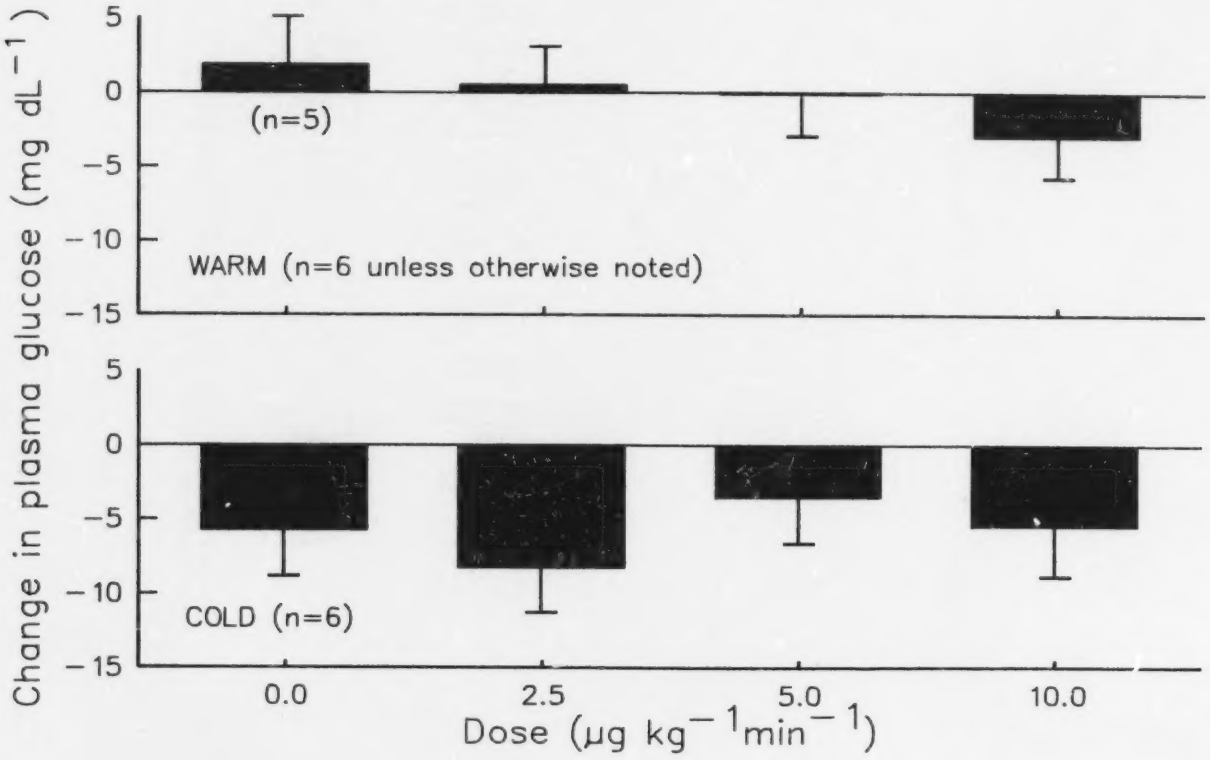


Figure 6: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma glucose in warm– and cold–acclimated wethers

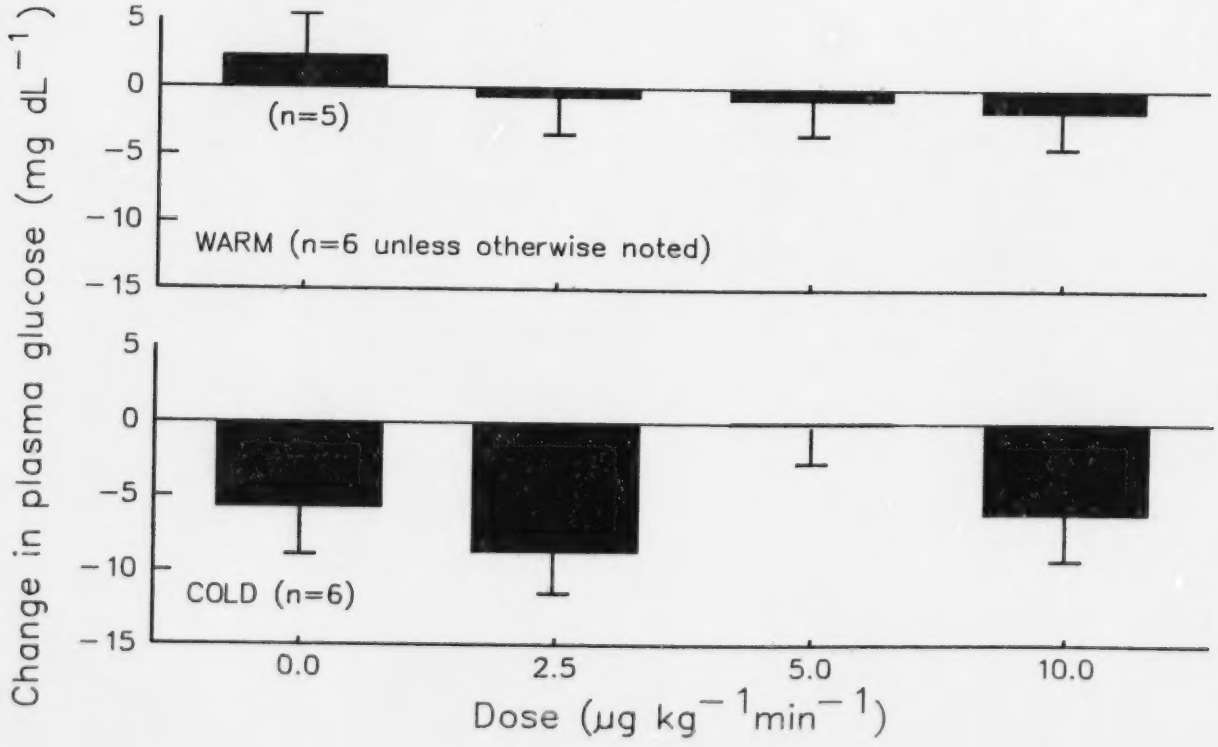


Figure 7: The effects of various 3–h infusions of metoprolol (B1) on plasma urea nitrogen (PUN) in warm– and cold–acclimated wethers

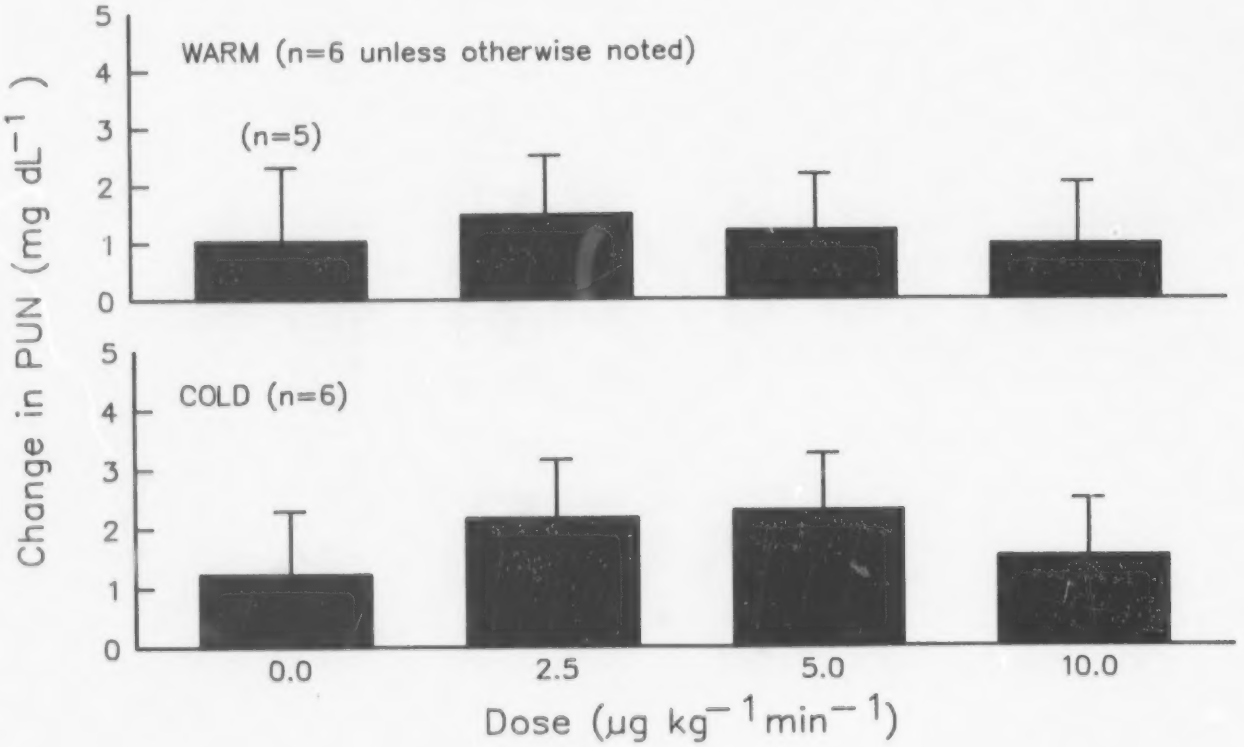


Figure 8: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma urea nitrogen (PUN) in warm– and cold–acclimated wethers

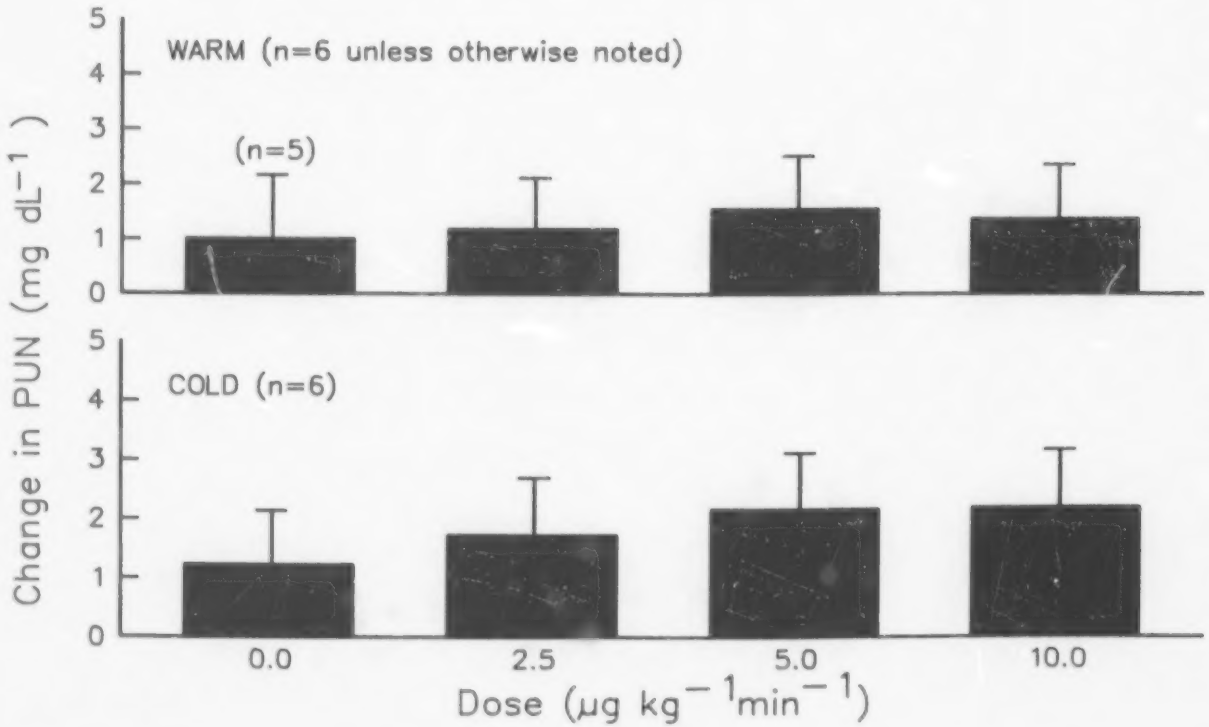


Figure 9: The effects of various 3–h infusions of metoprolol (B1) on plasma total amino acids in warm– and cold–acclimated wethers

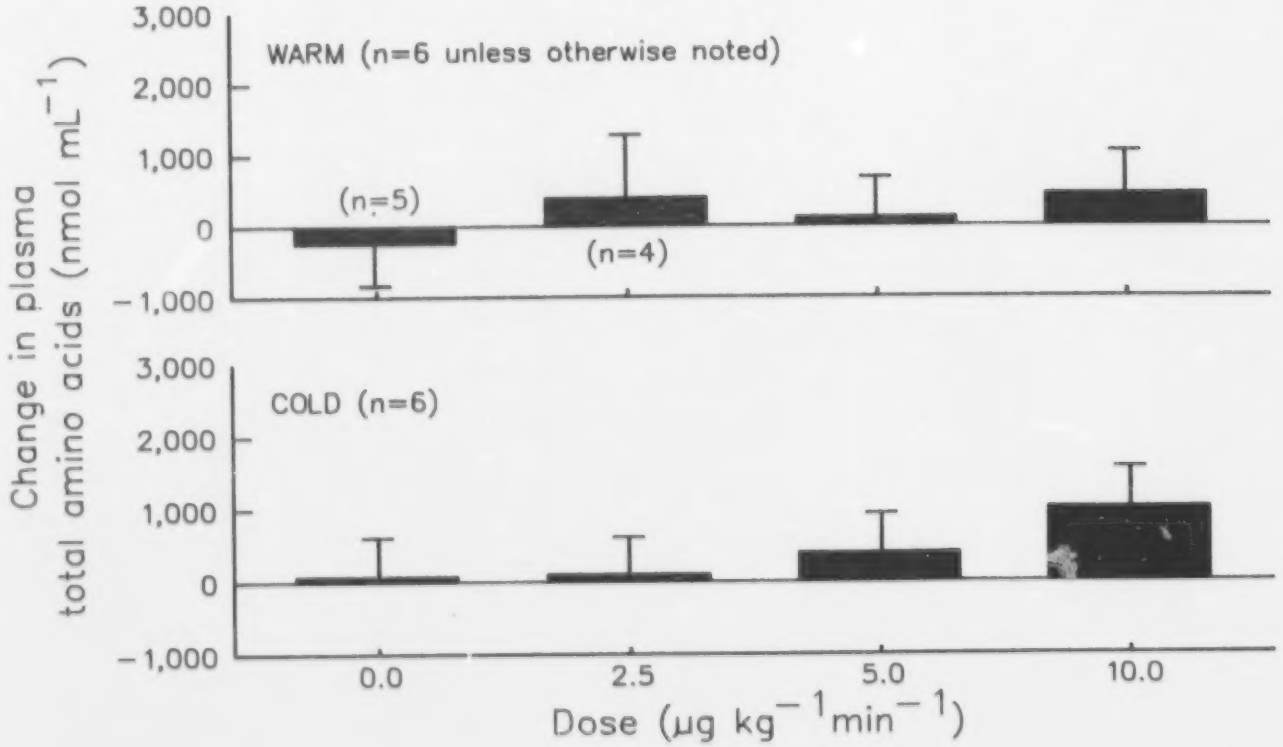


Figure 10: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma total amino acids in warm– and cold–acclimated wethers (* $P<0.06$)

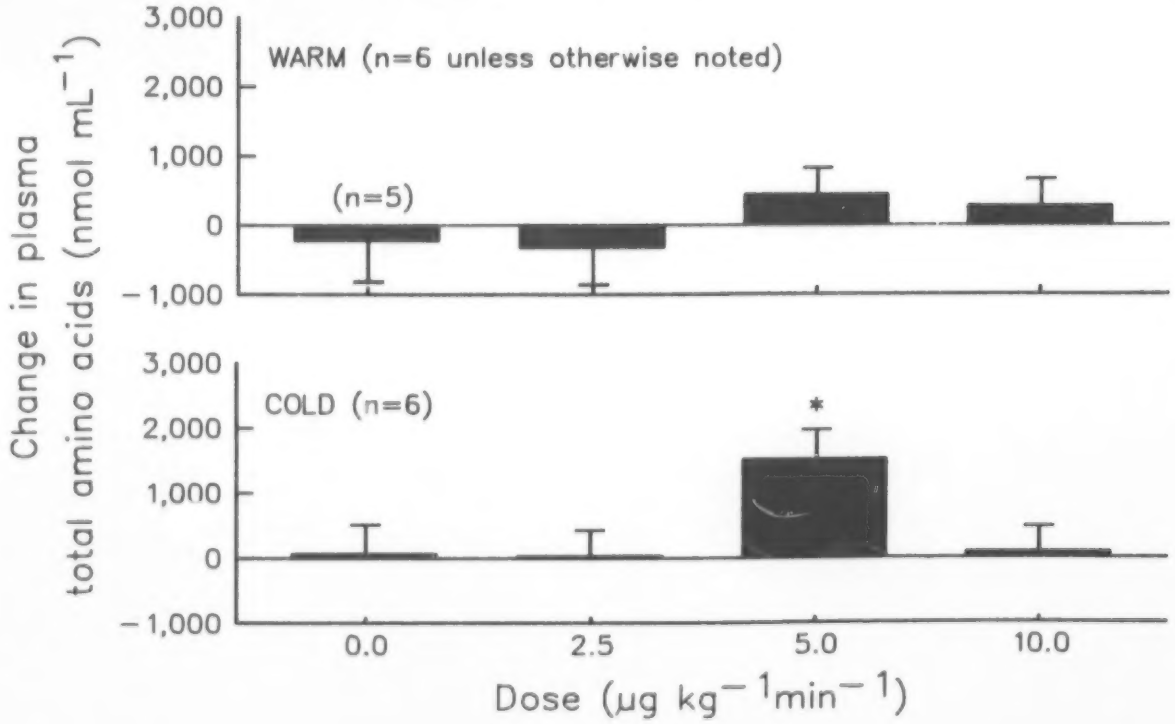


Figure 11: The effects of various 3–h infusions of metoprolol (B1) on plasma alanine in warm– and cold–acclimated wethers

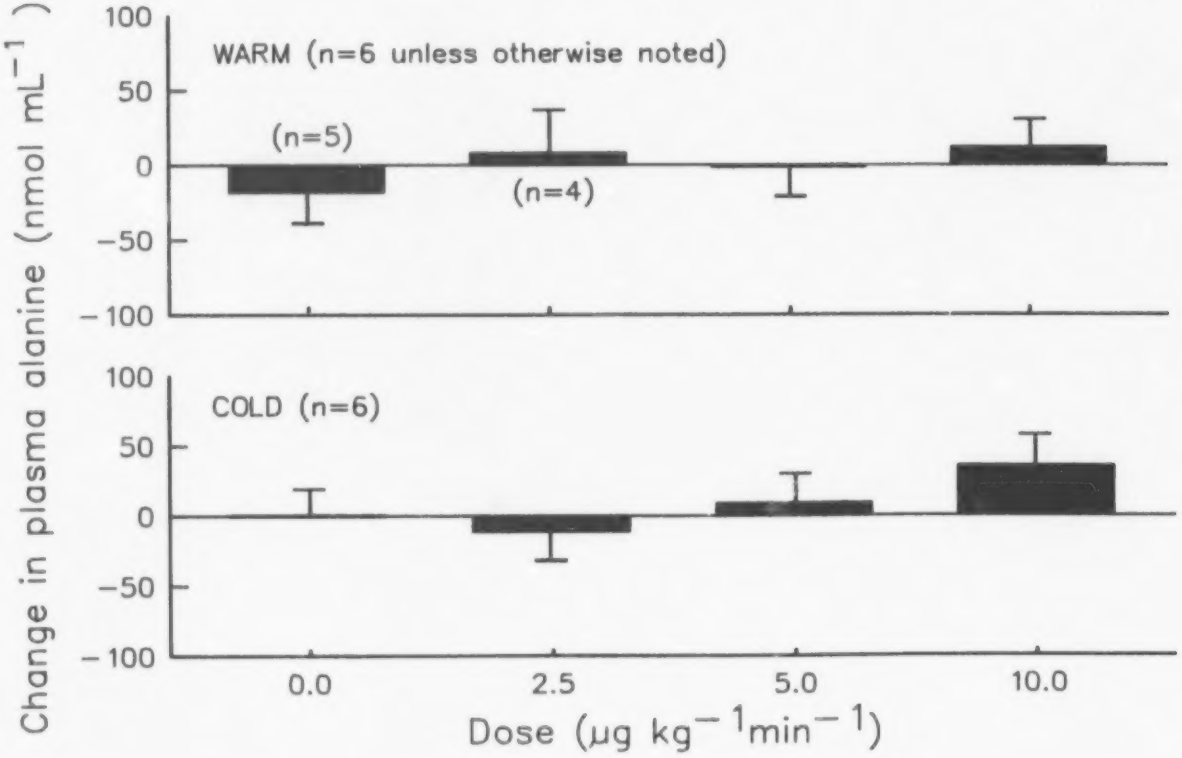


Figure 12: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma alanine in warm– and cold–acclimated wethers

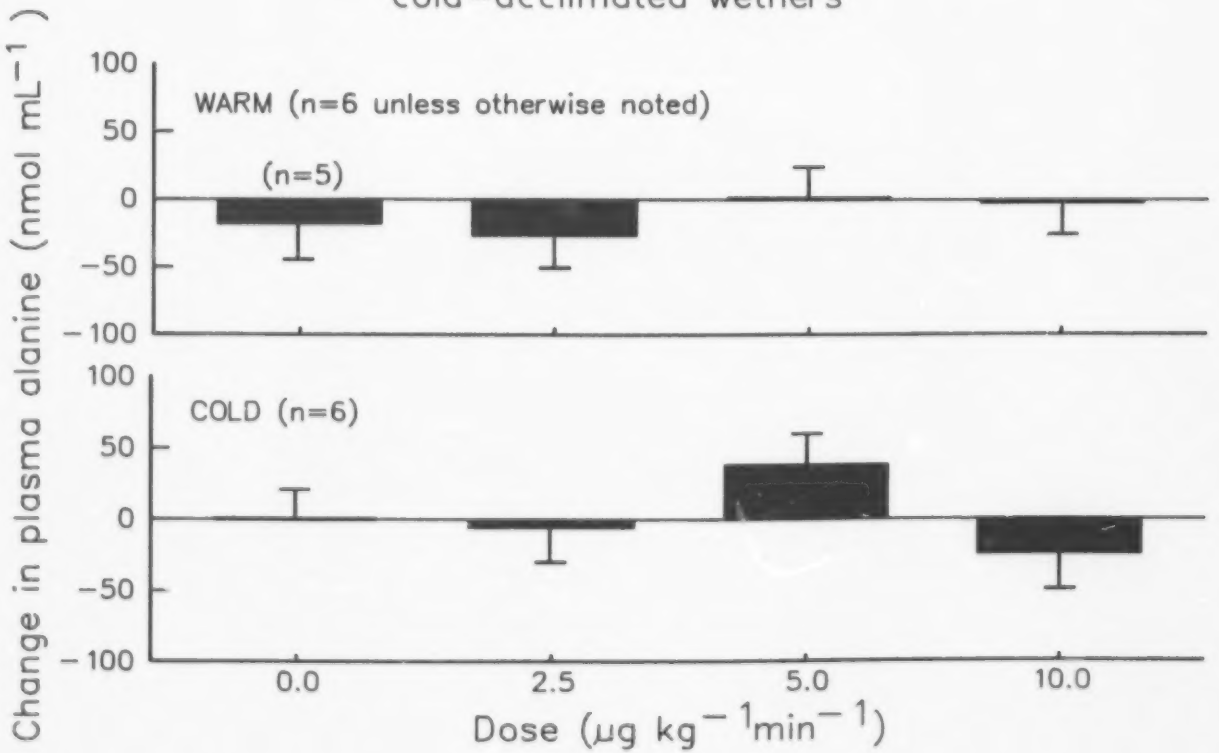


Figure 13: The effects of various 3–h infusions of metoprolol (B1) on plasma glutamine in warm– and cold–acclimated wethers (* P<0.02)

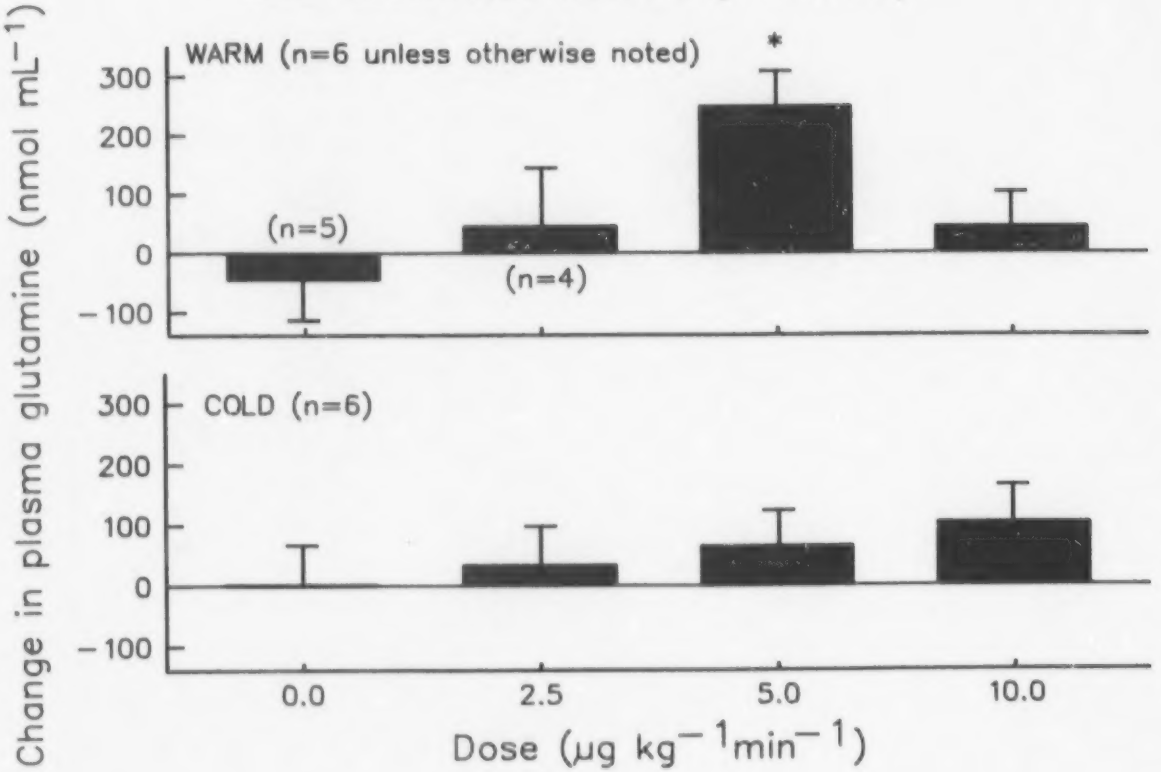


Figure 14: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma glutamine in warm– and cold–acclimated wethers (* P<0.04)

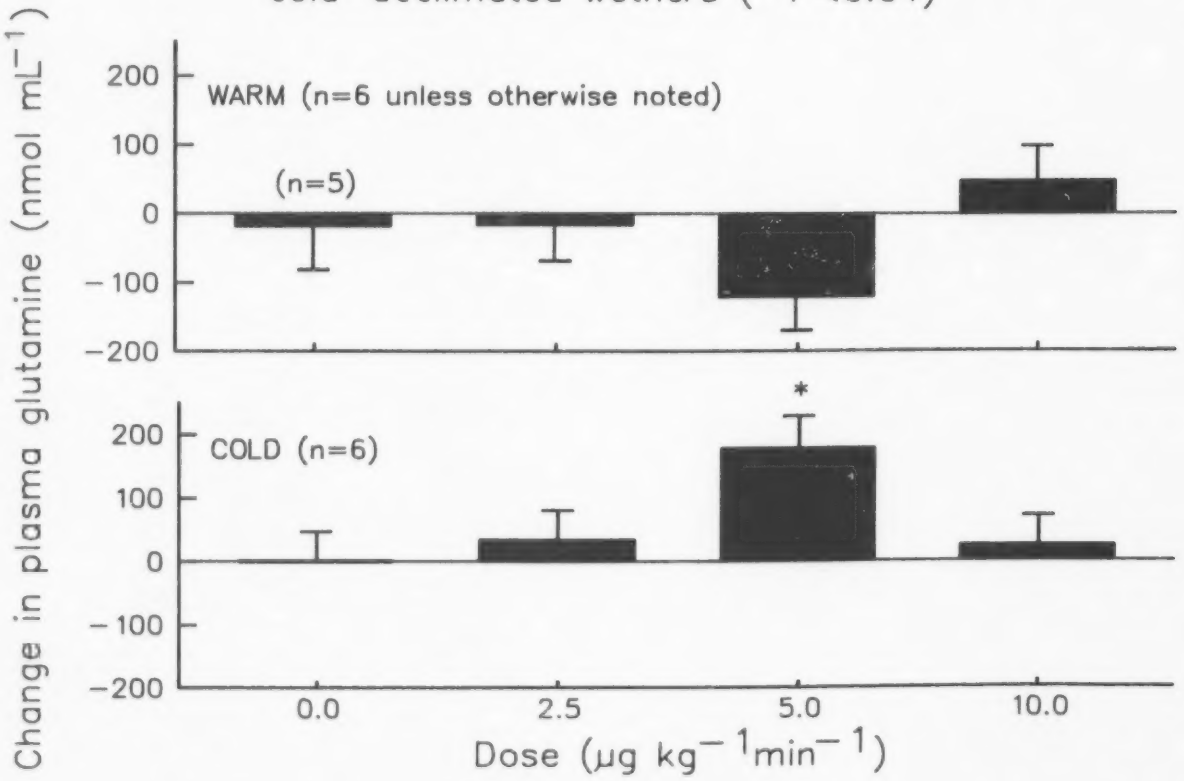


Figure 15: The effects of various 3–h infusions of metoprolol (B1) on plasma valine in warm– and cold–acclimated wethers

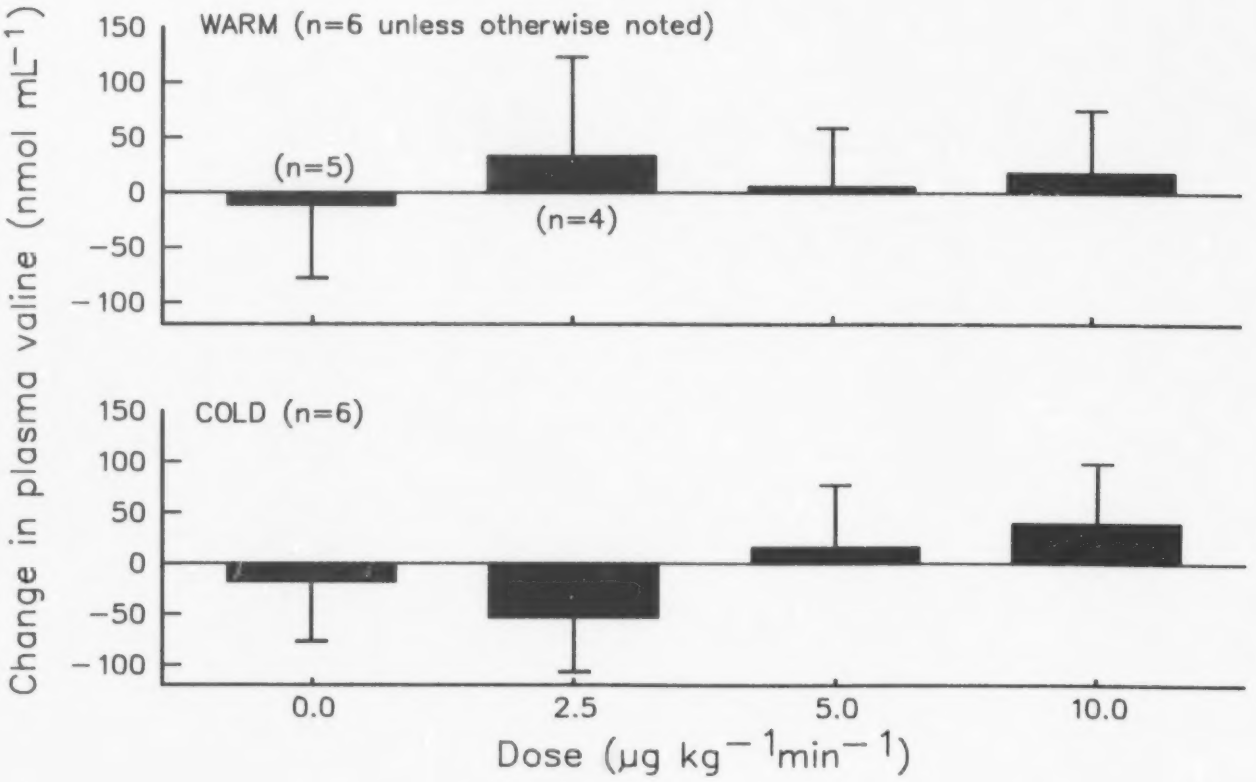


Figure 16: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma valine in warm– and cold–acclimated wethers (* P<0.03)

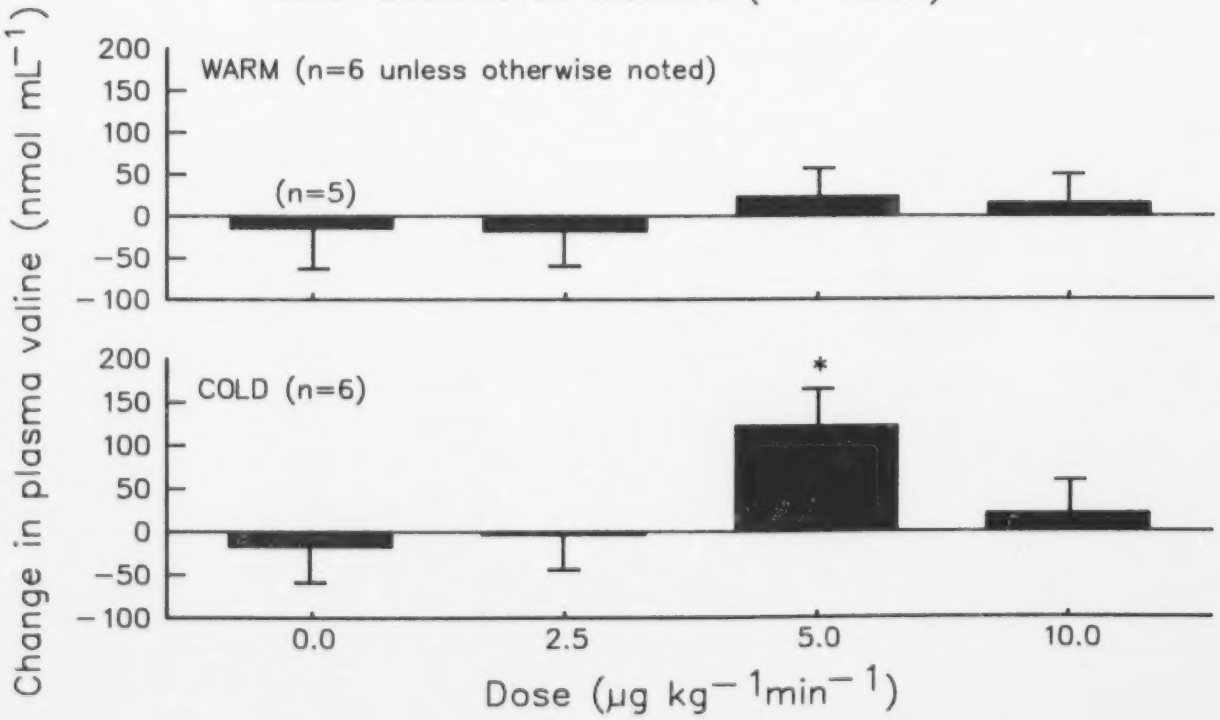


Figure 17: The effects of various 3–h infusions of metoprolol (B 1) on plasma leucine in warm– and cold–acclimated wethers

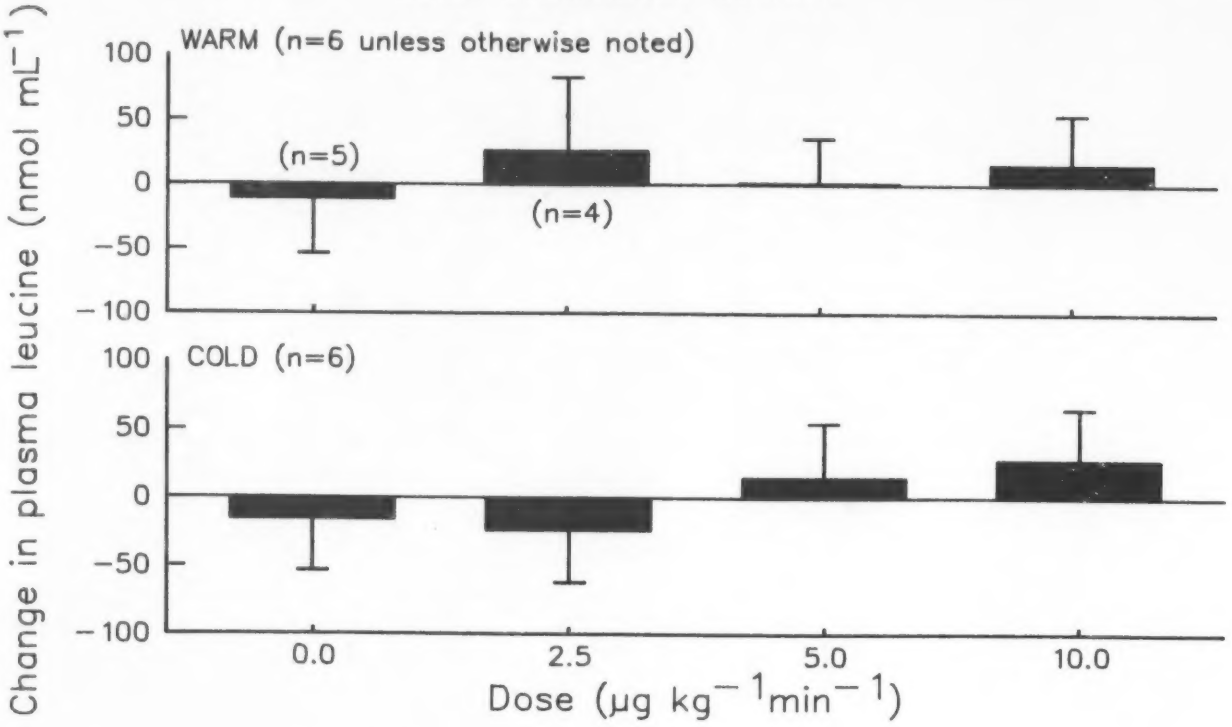


Figure 18: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma leucine in warm– and cold–acclimated wethers (* P<0.02)

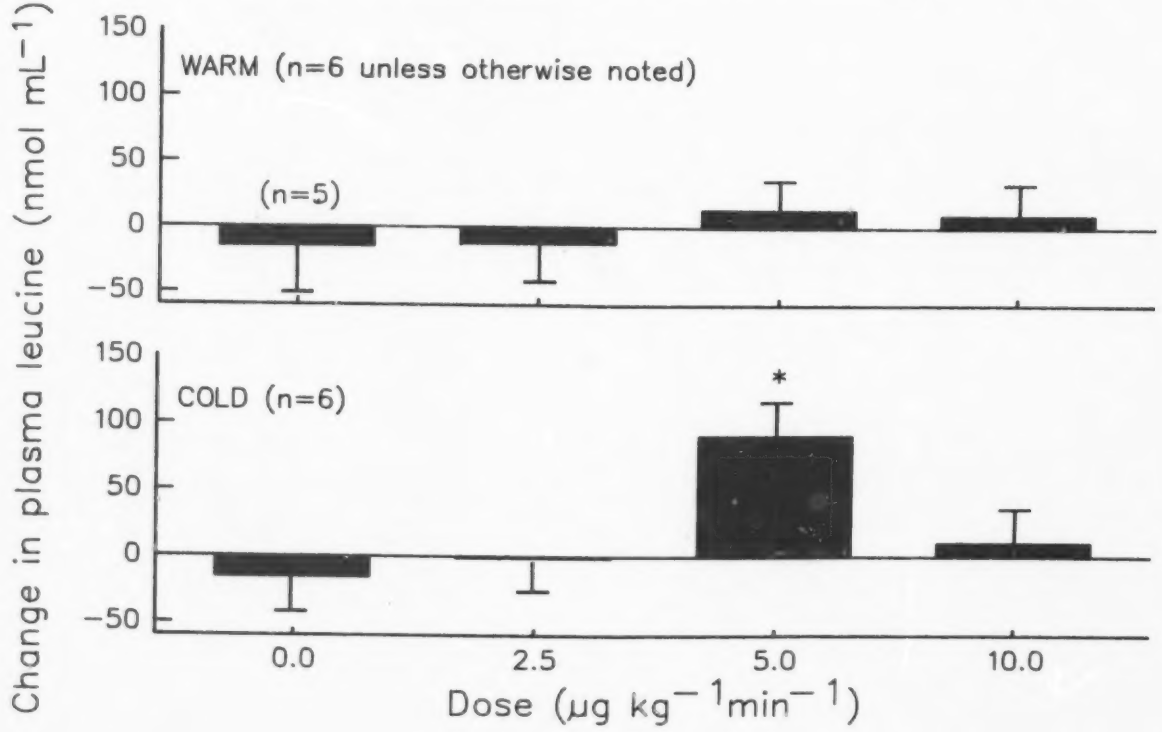


Figure 19: The effects of various 3–h infusions of metoprolol (B1) on plasma isoleucine in warm– and cold–acclimated wethers

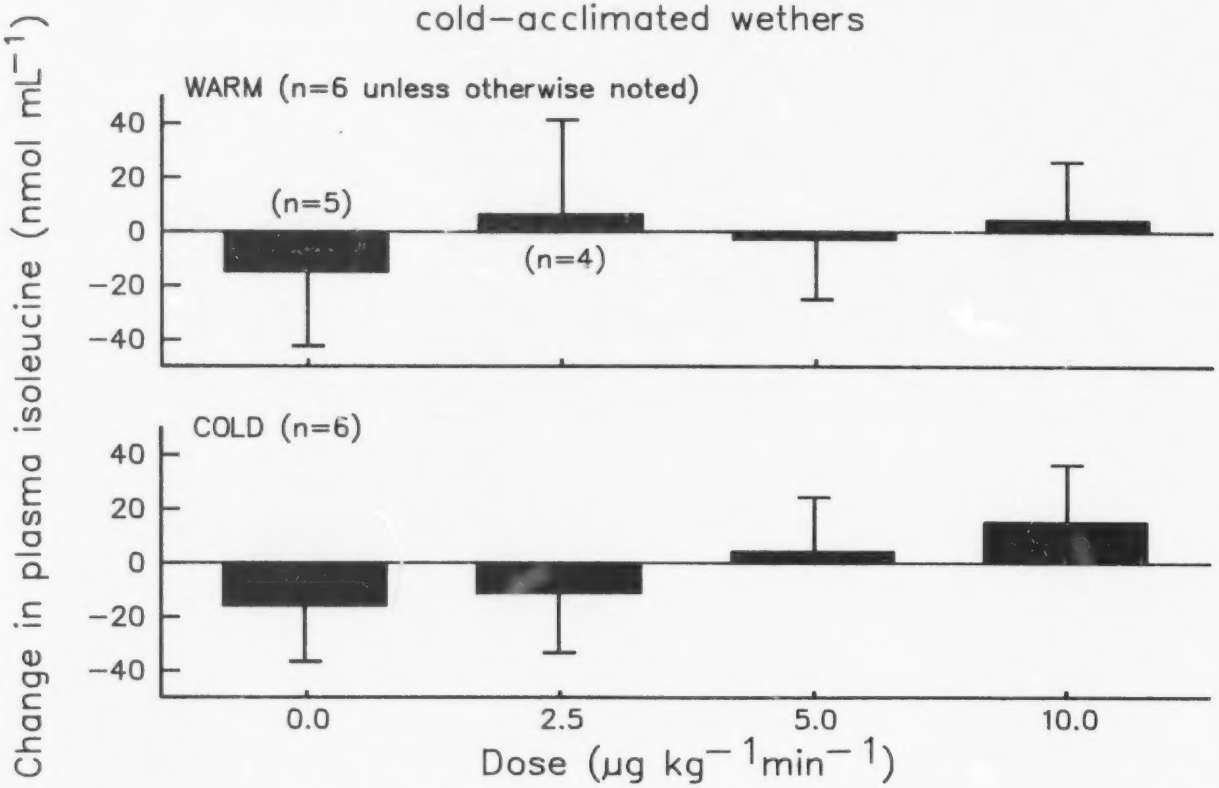


Figure 20: The effects of various 3–h infusions of ICI 118,551 (B2) on plasma isoleucine in warm– and cold–acclimated wethers (* P<0.03)

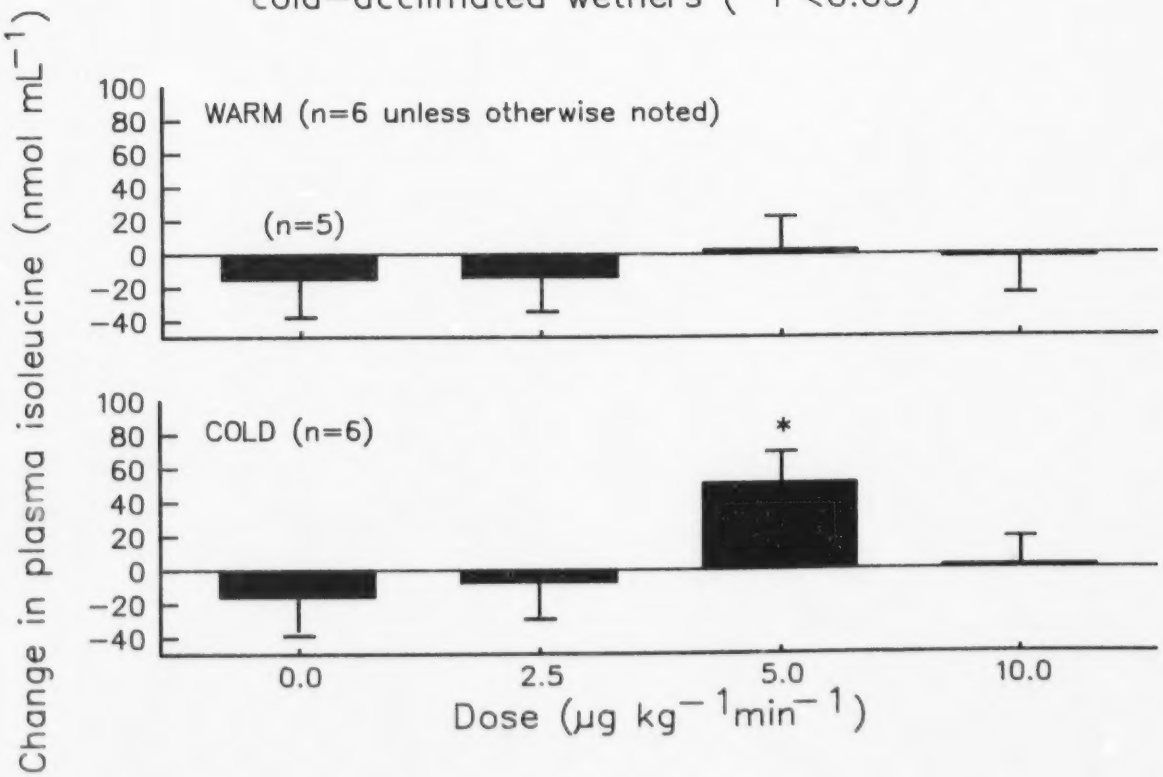


Figure 21: Dose response of heat production (HP) to ICI 118,551 (B2) in warm– and cold–acclimated wethers

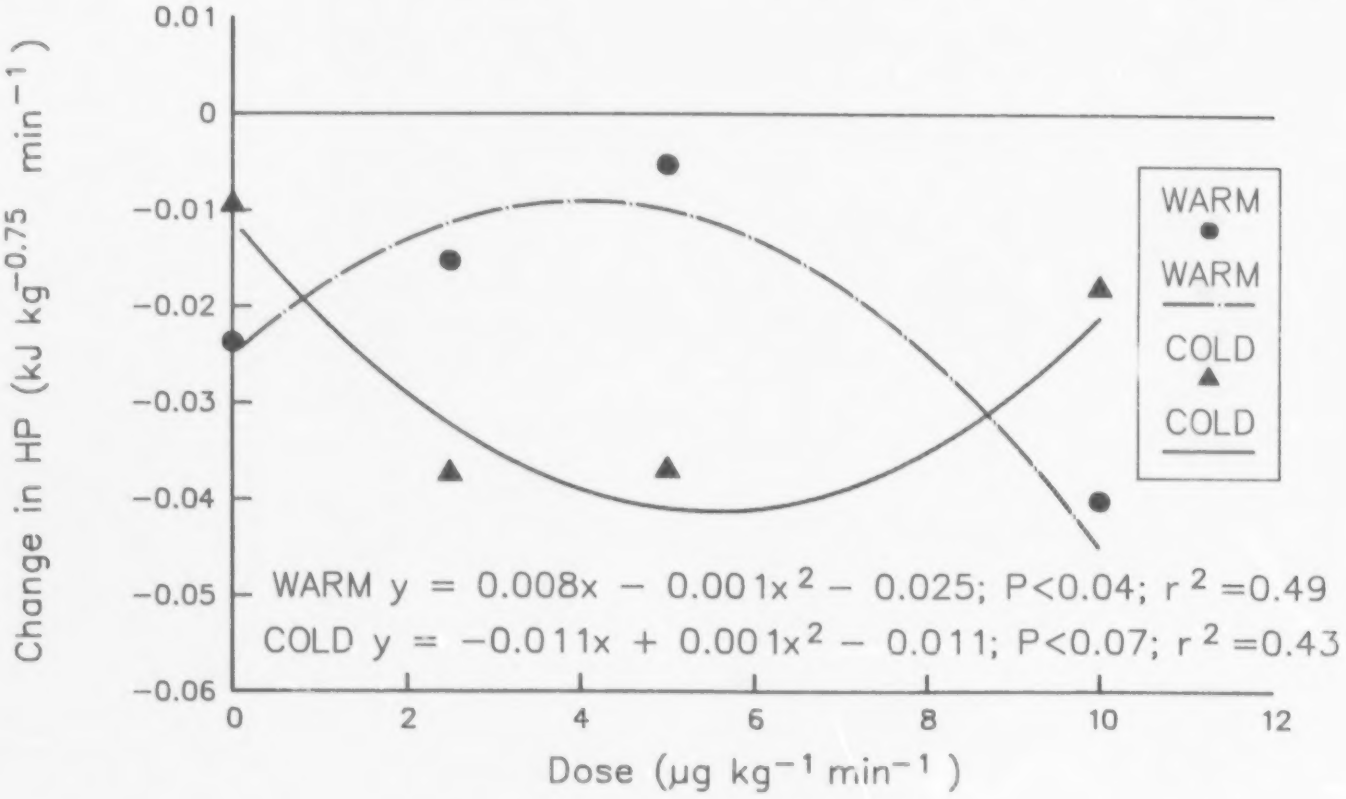


Figure 22: Dose response of plasma glycerol to metoprolol (B1) in warm– and cold–acclimated wethers

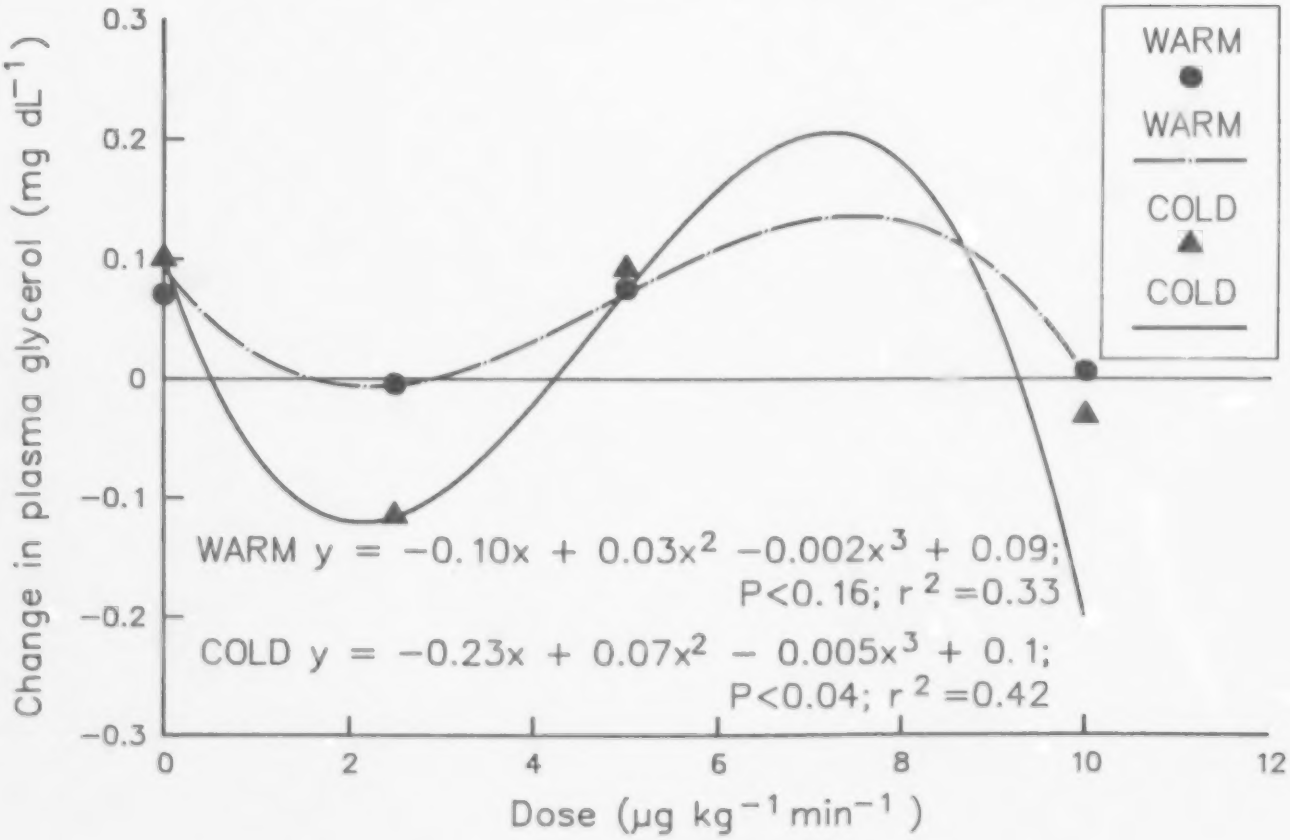


Figure 23: Dose response of plasma glycerol to ICI 118,551 (B2) in warm- and cold-acclimated wethers

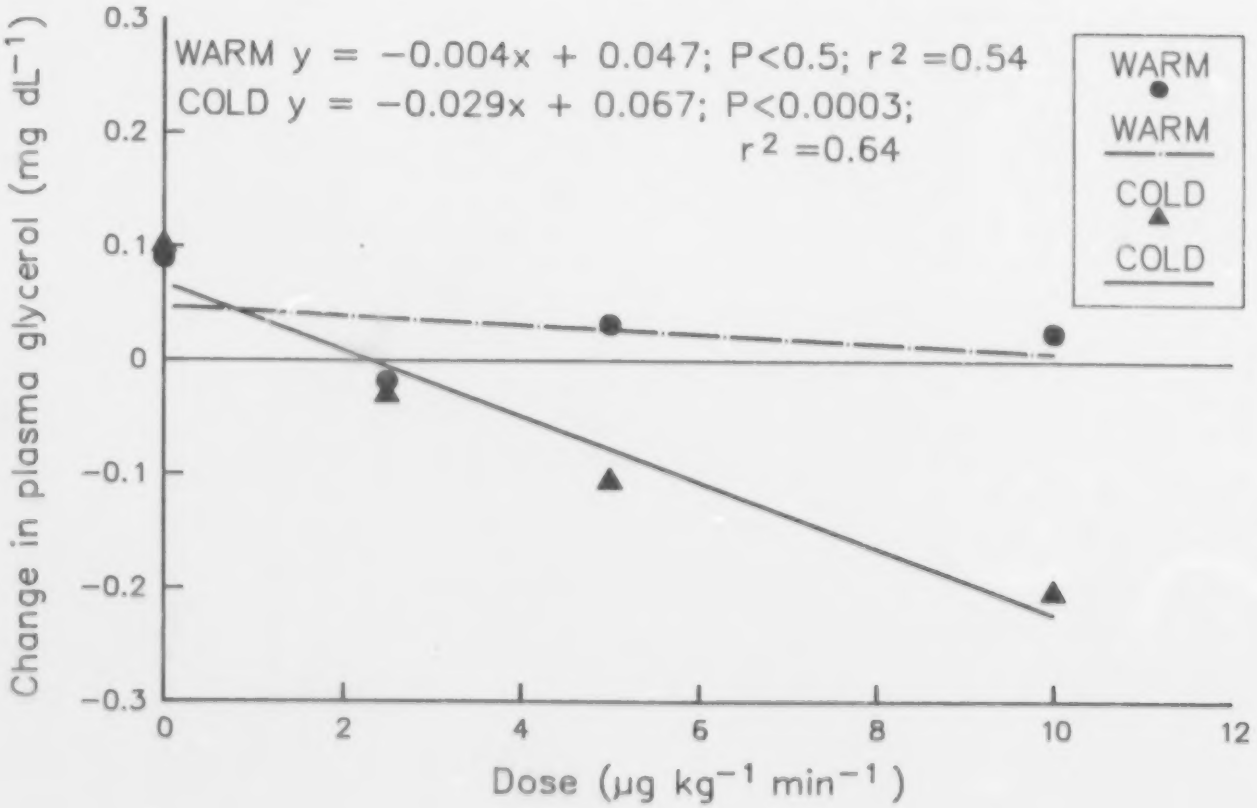


Figure 24: Dose response of plasma glutamine to metoprolol (B1) in warm– and cold–acclimated wethers

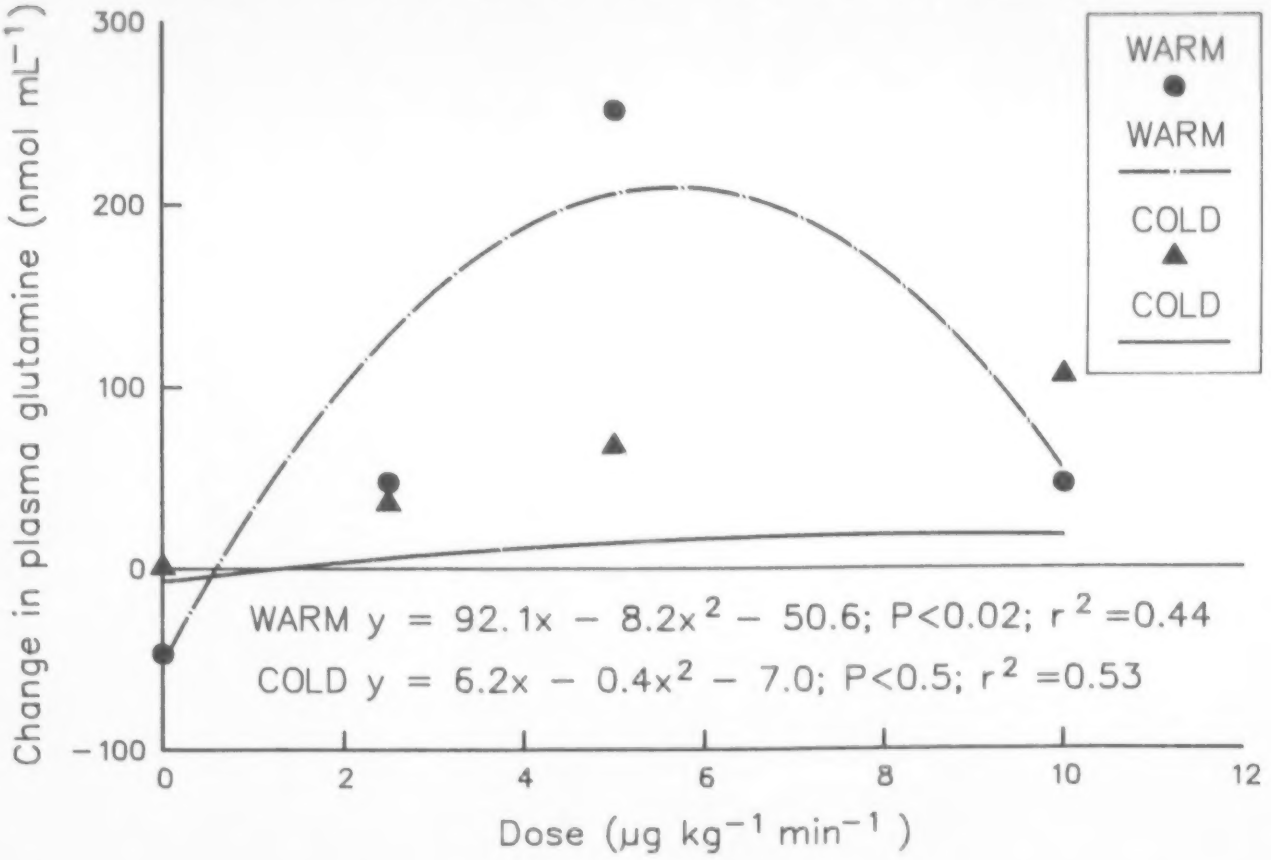


Figure 25: Dose response of plasma glutamine to ICI 118,551 (B2) in warm– and cold–acclimated wethers

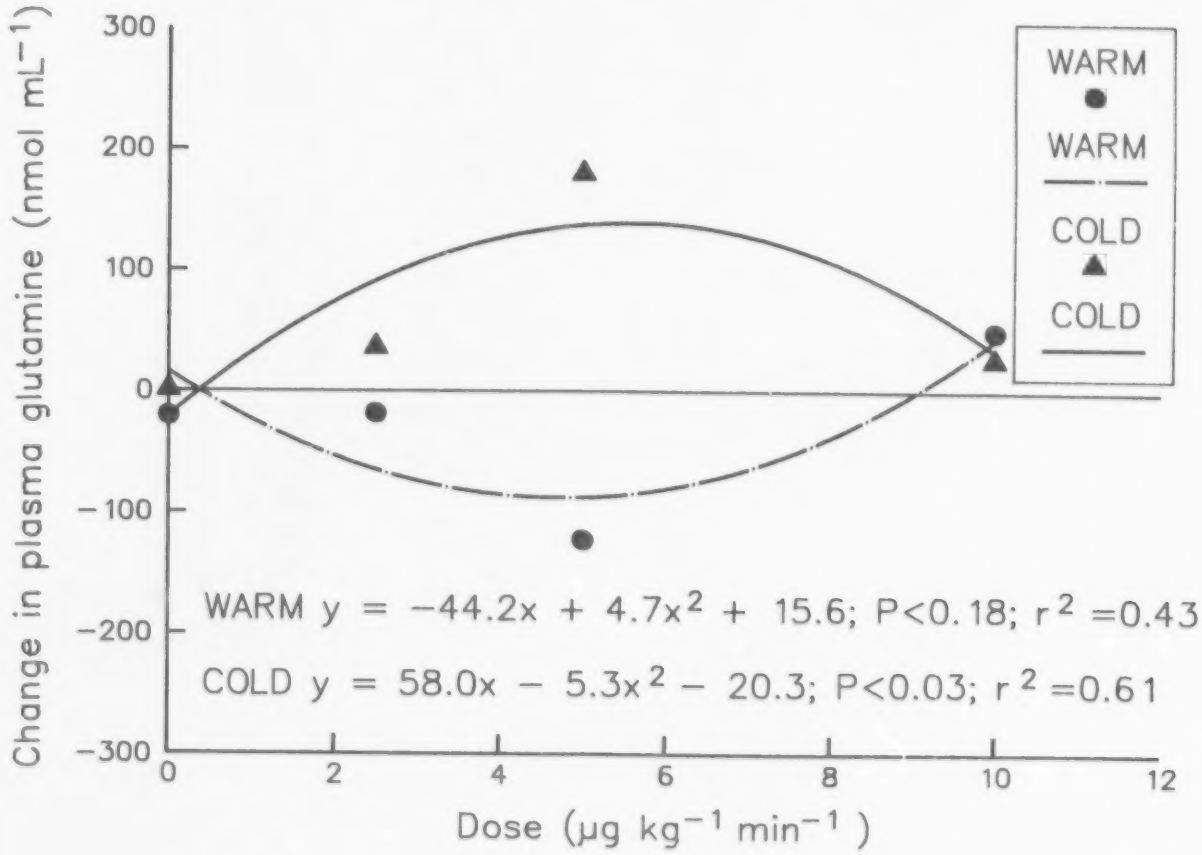
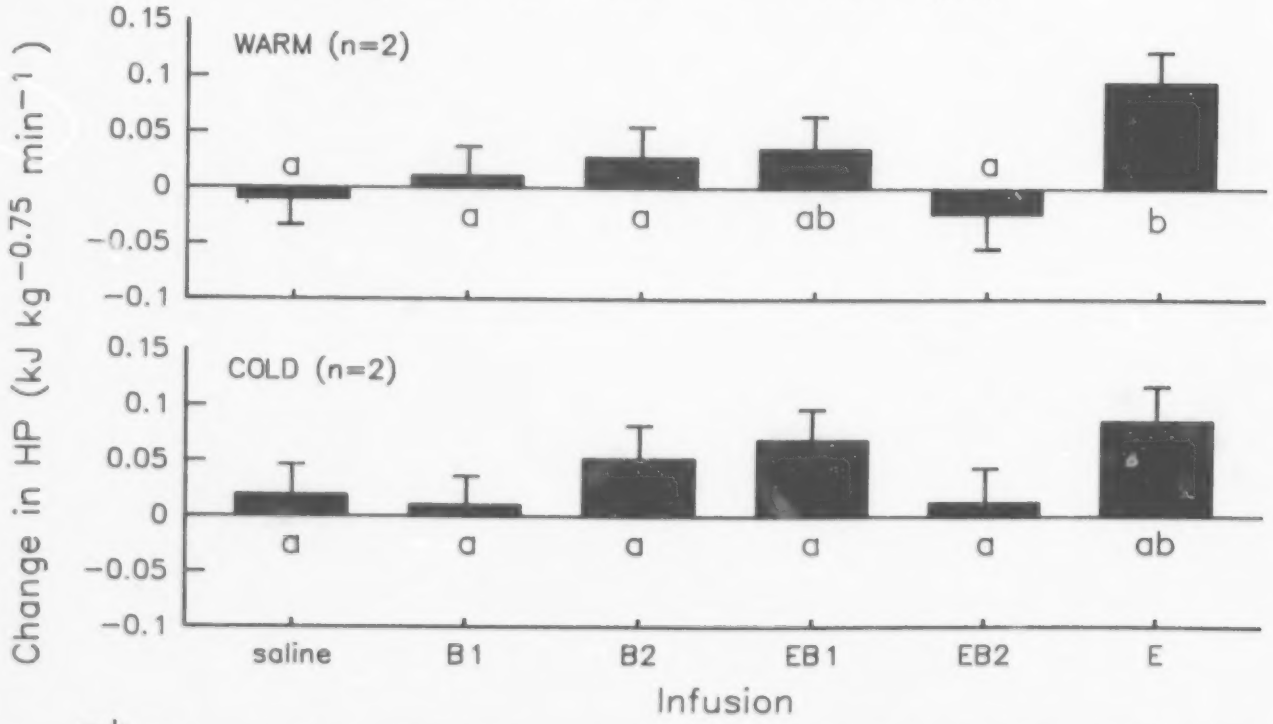
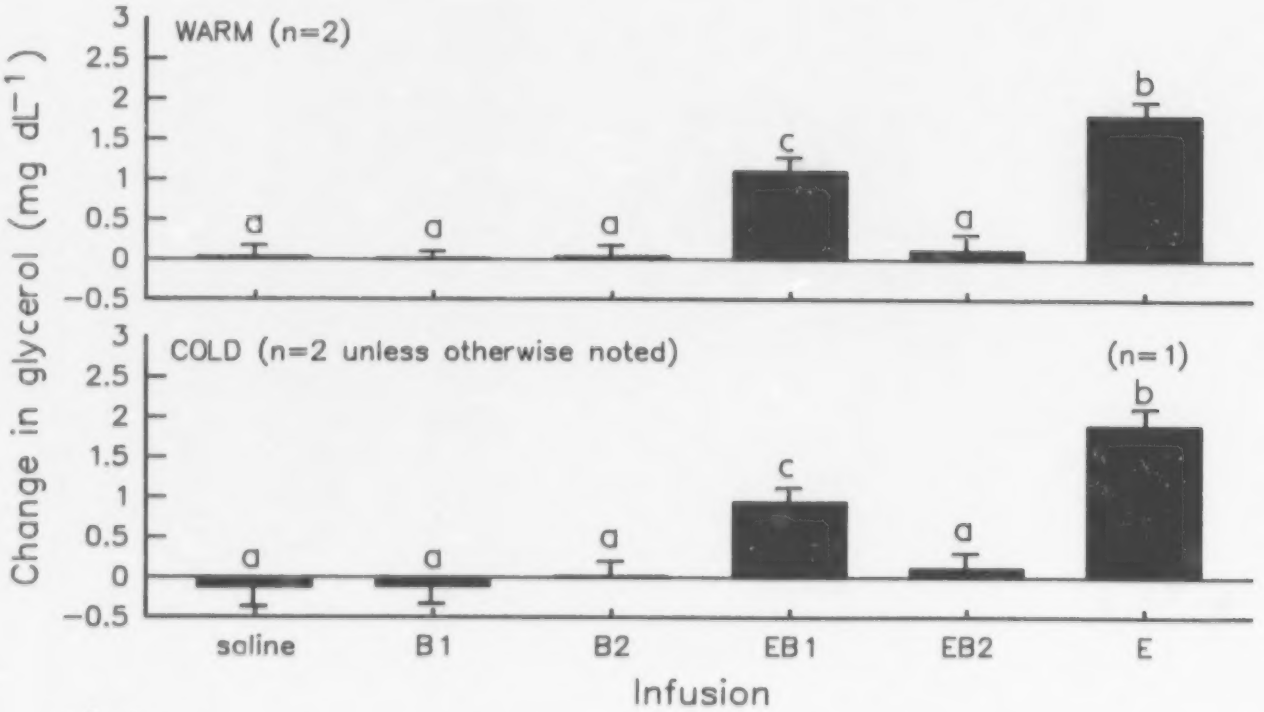


Figure 26: The response of heat production (HP) in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



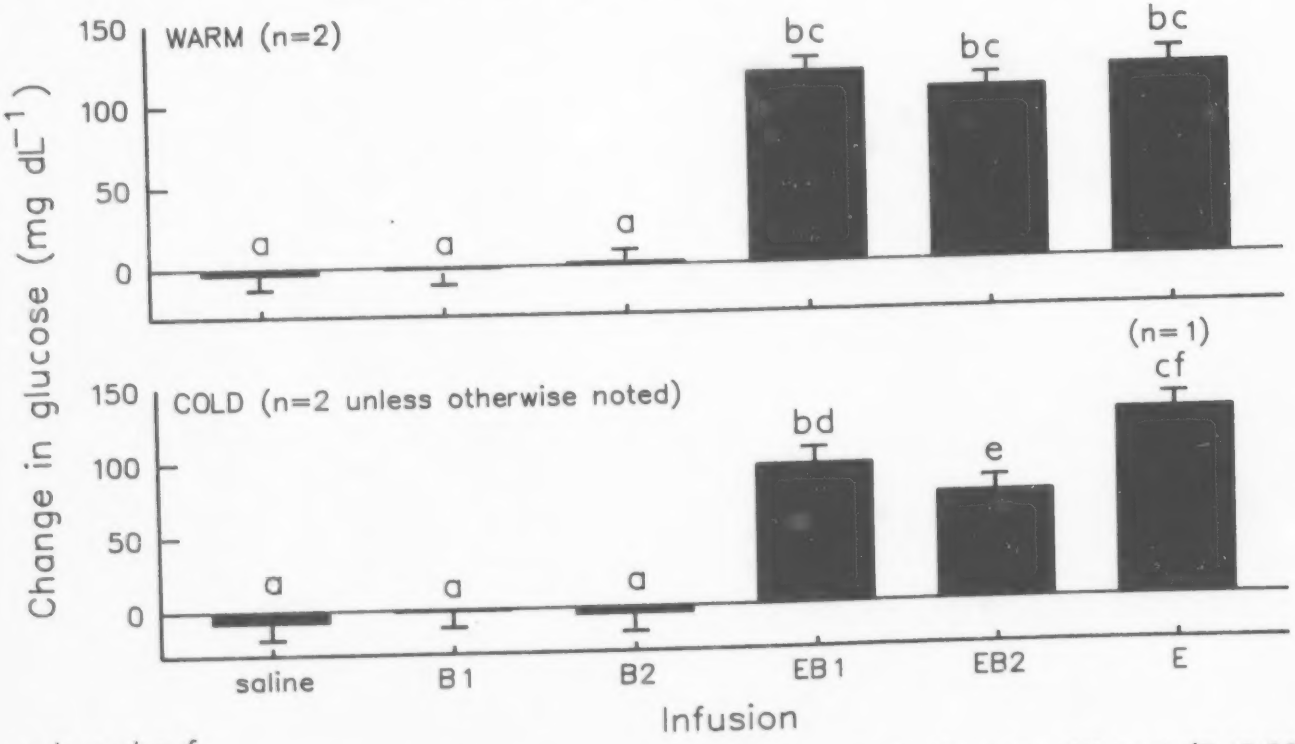
a,b LS means with different lower case letters are significantly different ($P<0.05$)

Figure 27: The response of plasma glycerol in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



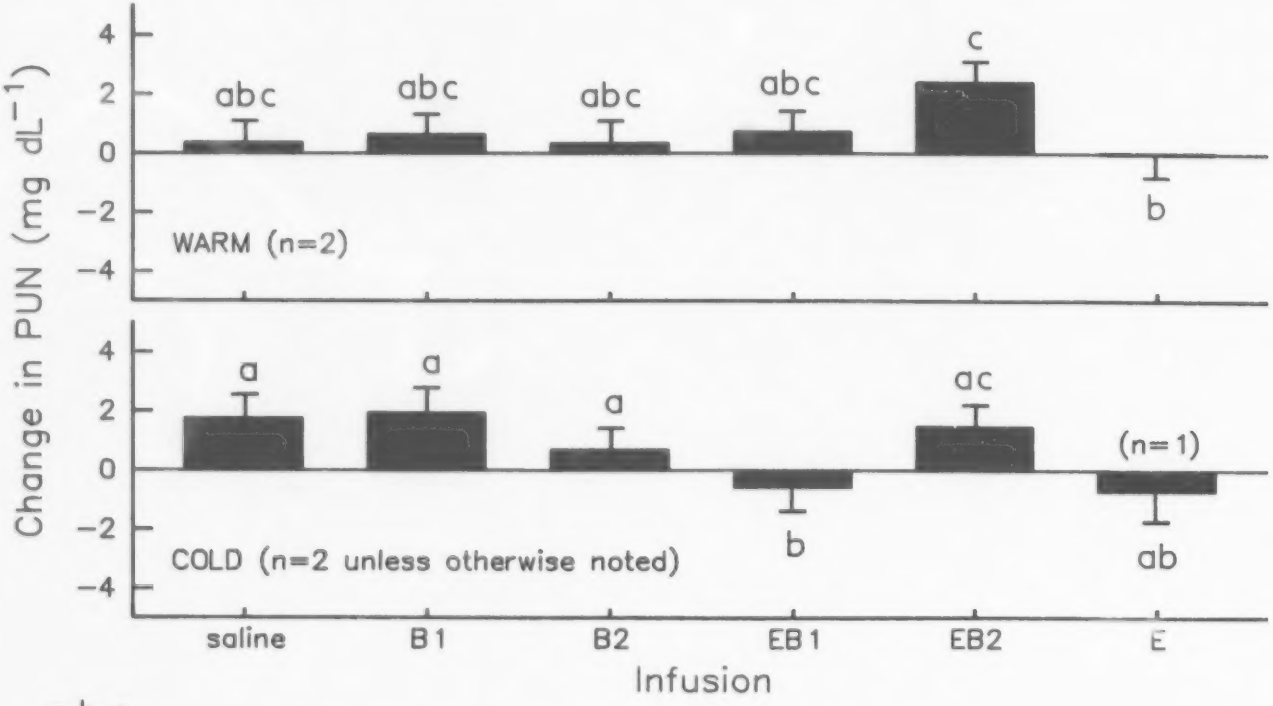
a,b,c LS means with different lower case letters are significantly different ($P<0.05$)

Figure 28: The response of plasma glucose in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



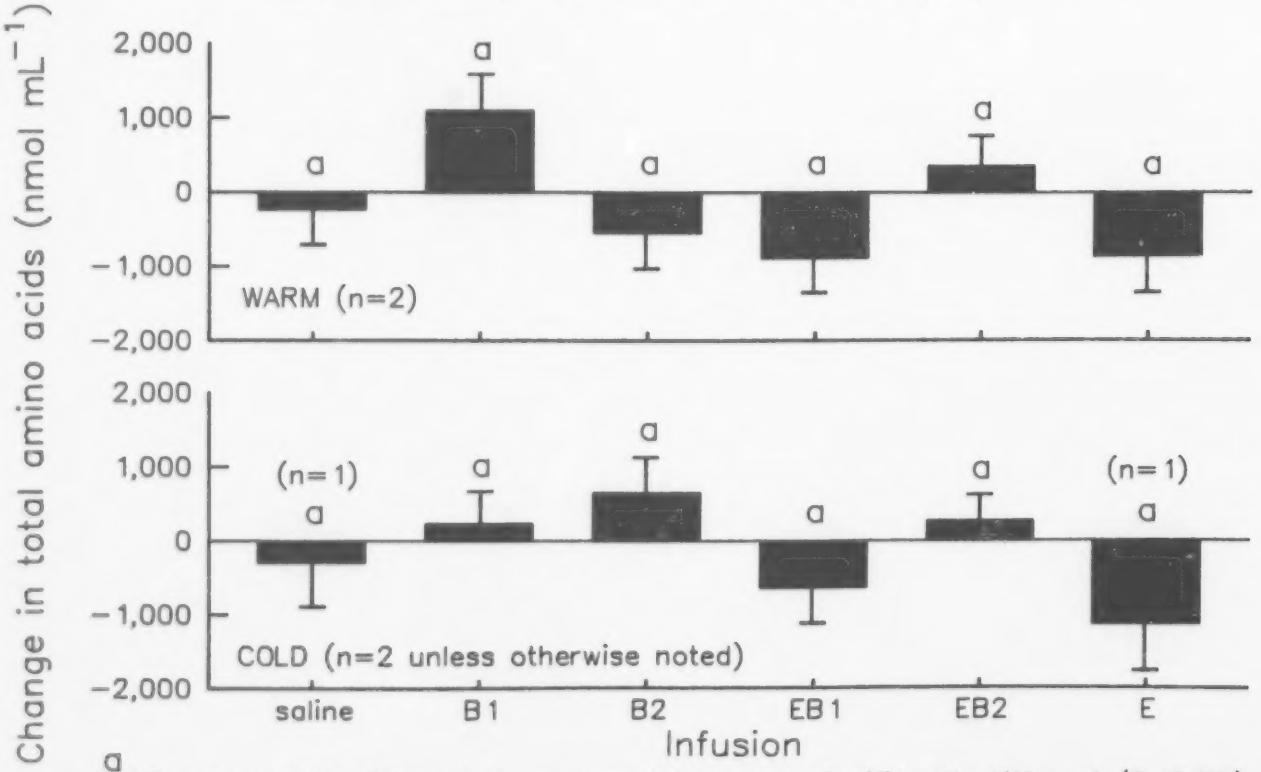
a,b,c,d,e,f LS means with different lower case letters are significantly different ($P<0.05$)

Figure 29: The response of plasma urea nitrogen (PUN) in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



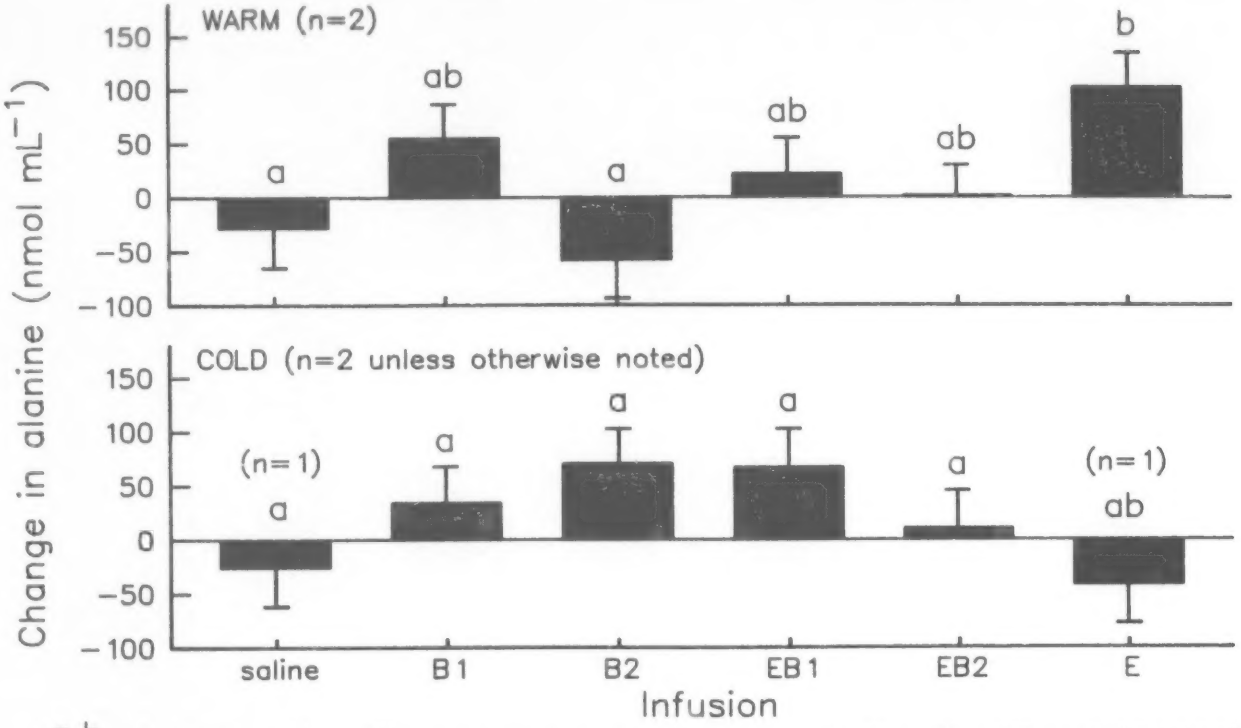
a,b,c LS means with different lower case letters are significantly different ($P<0.05$)

Figure 30: The response of plasma total amino acids in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



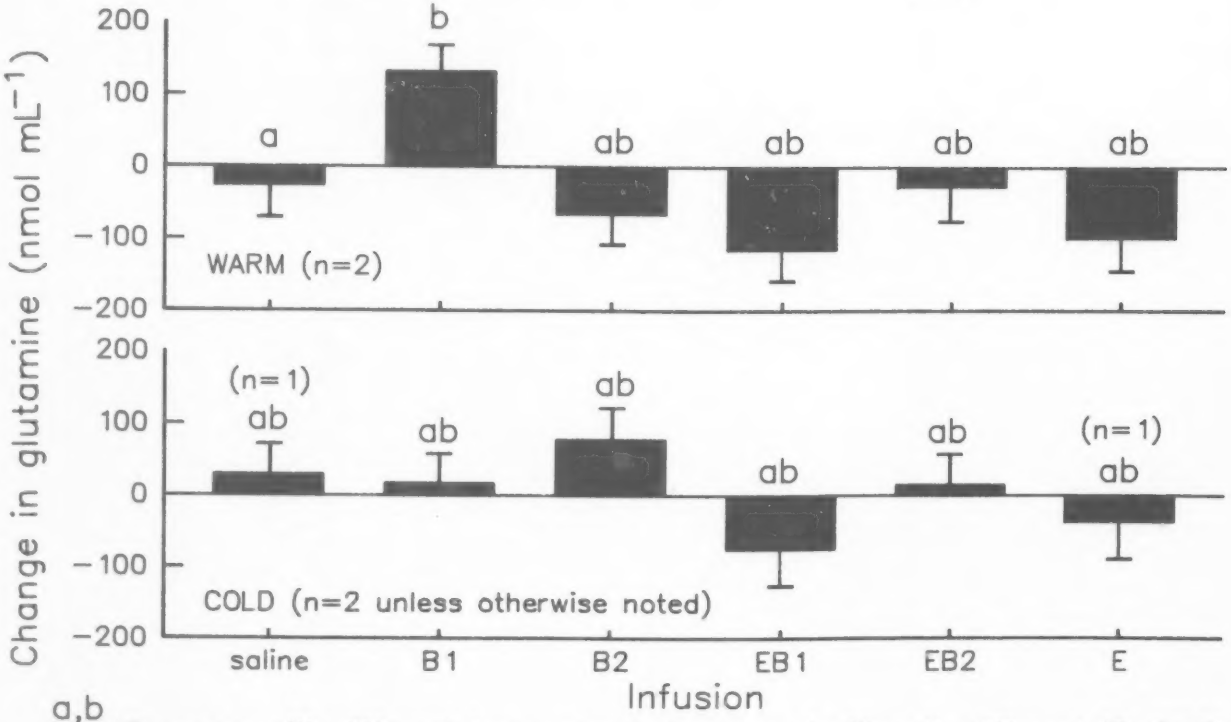
a LS means with different lower case letters are significantly different ($P<0.05$)

Figure 31: The response of plasma alanine in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



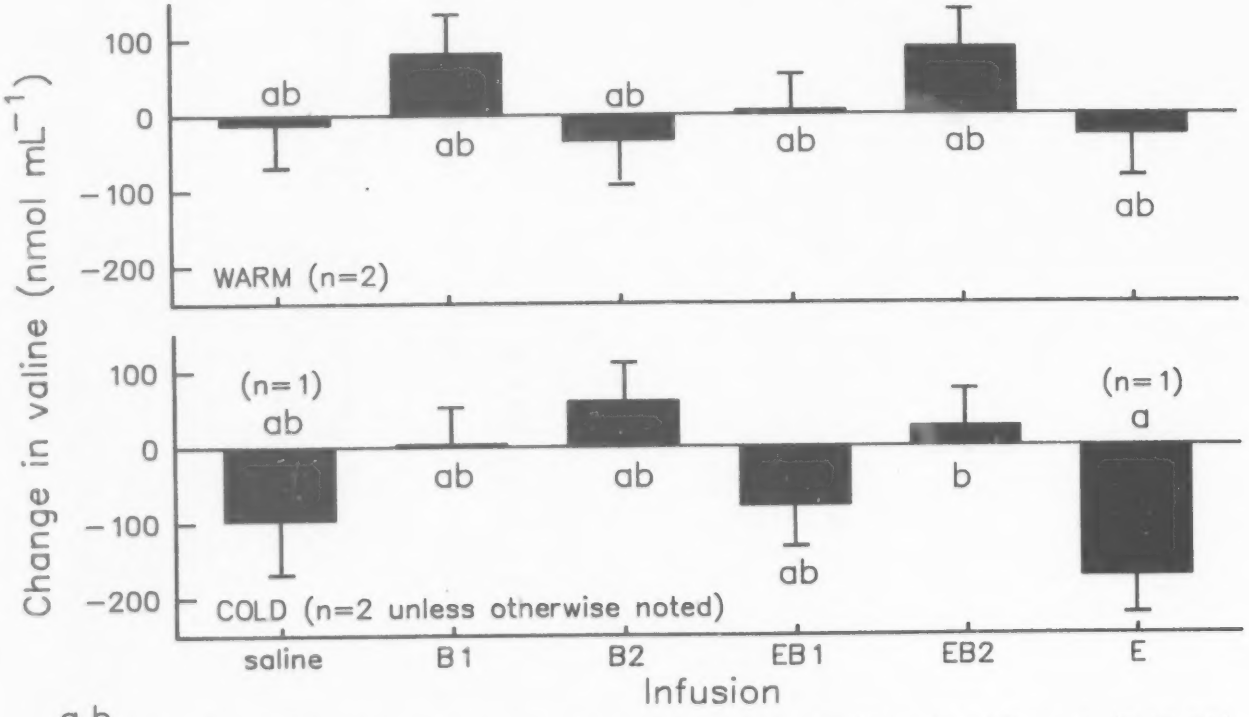
a,b LS means with different lower case letters are significantly different ($P<0.05$)

Figure 32: The response of plasma glutamine in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



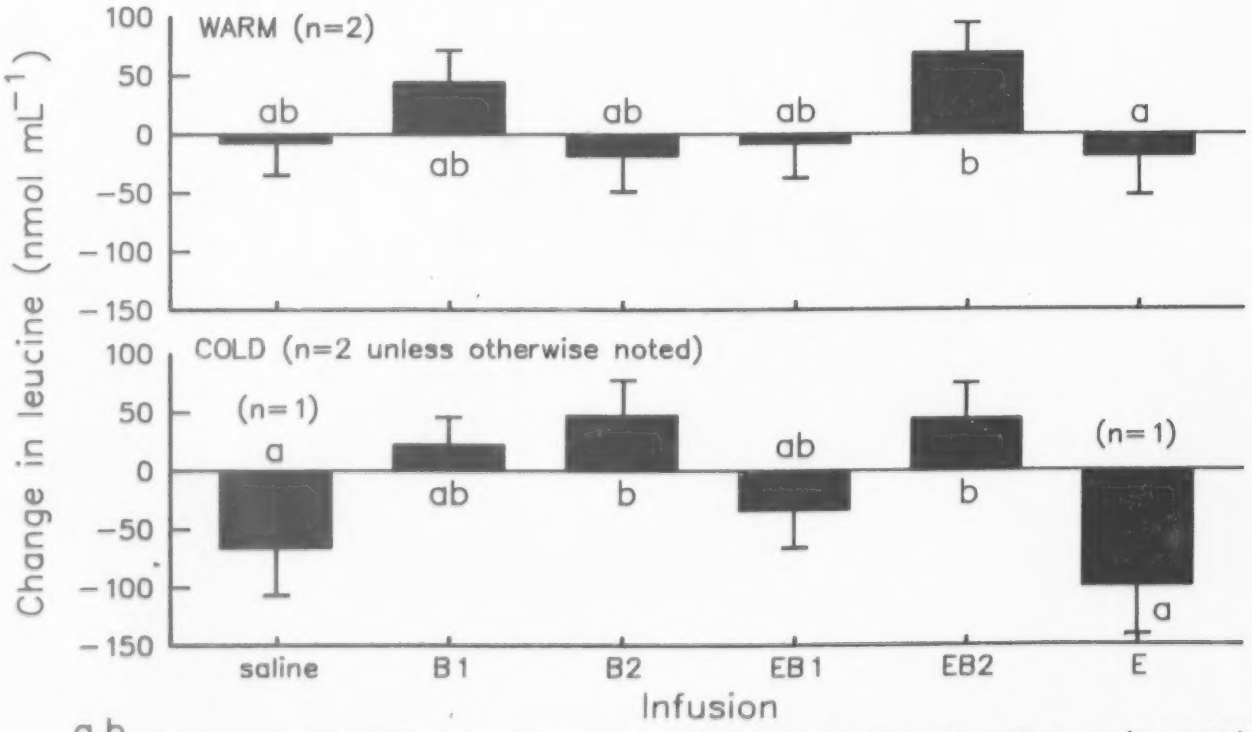
a,b LS means with different lower case letters are significantly different ($P<0.05$)

Figure 33: The response of plasma valine in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



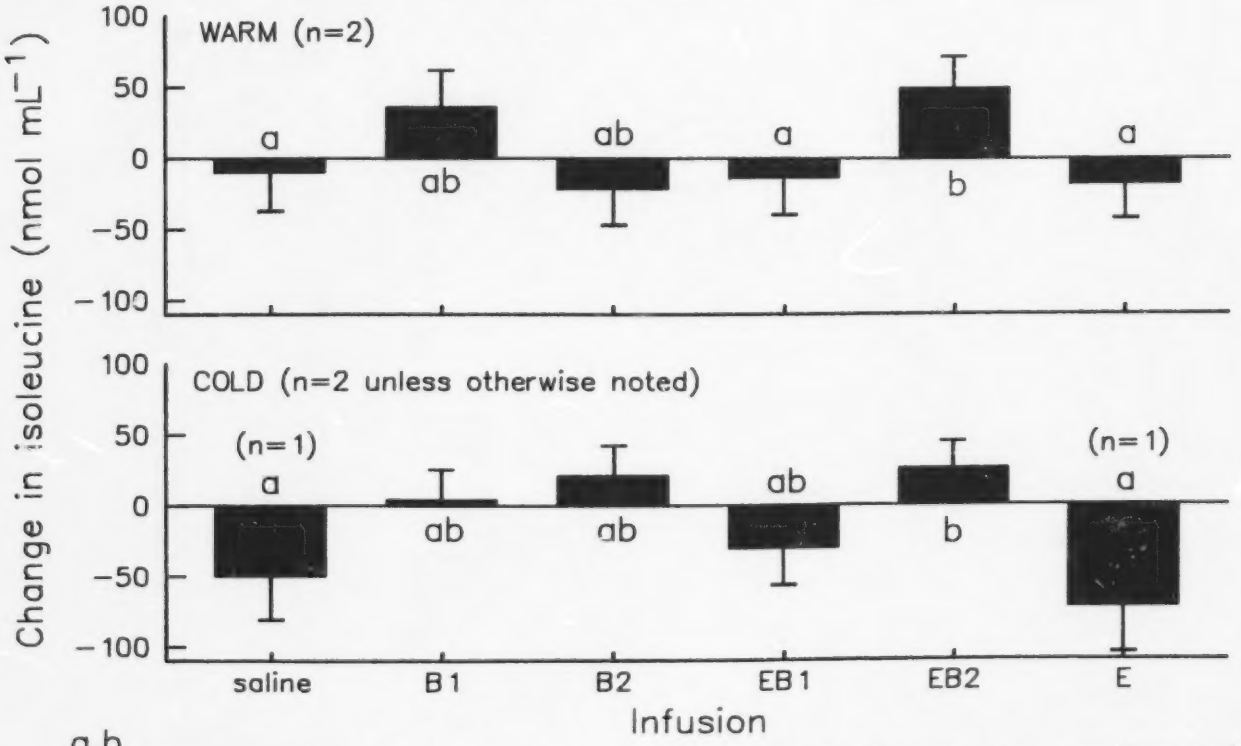
a,b LS means with different lower case letters are significantly different ($P<0.05$)

Figure 34: The response of plasma leucine in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



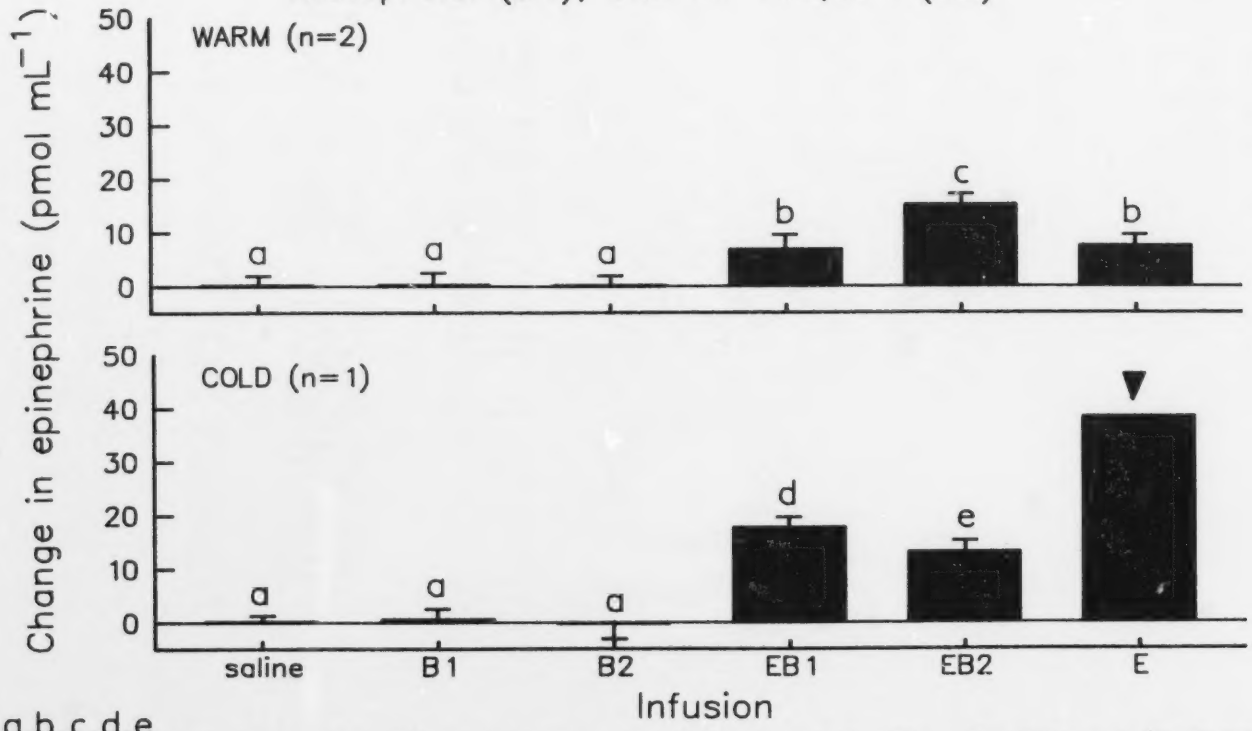
a,b LS means with different lower case letters are significantly different ($P<0.05$)

Figure 35: The response of plasma isoleucine in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



a,b LS means with different lower case letters are significantly different ($P<0.05$)

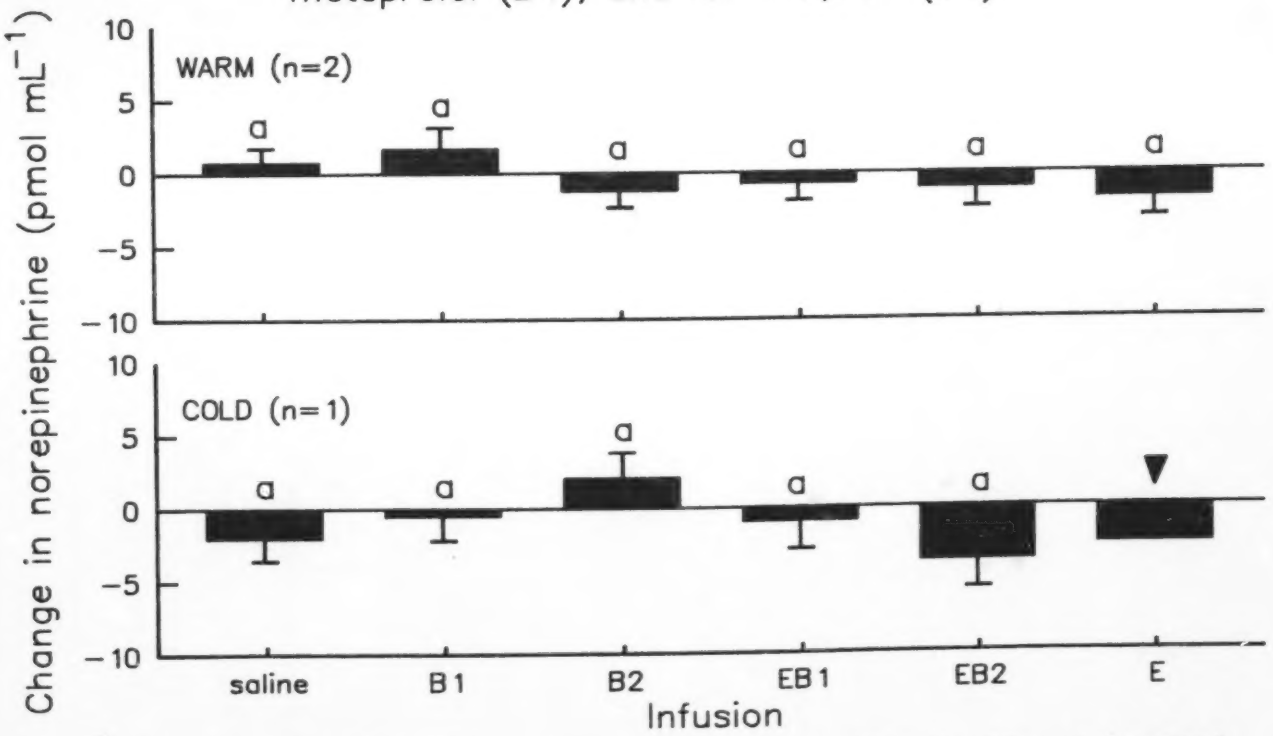
Figure 36: The response of plasma epinephrine in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



a,b,c,d,e LS means with different lower case letters are significantly different ($P<0.05$)

▼ Change was calculated using average of other cold preinfusion values

Figure 37: The response of plasma norepinephrine in warm– and cold–acclimated wethers to combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



a LS means with different lower case letters are significantly different ($P<0.05$)

▼ Change was calculated using average of other cold preinfusion values

DISCUSSION

EXPERIMENT ONE

Effects of Temperature. There was a significant effect of cold-acclimation on rectal temperature, as the CA animals' mean rectal temperature was lower than that in the WA animals (Table 2). Herpin et al. (1987) proposed that pigs may adapt to the cold by slightly decreasing their body temperature as a mechanism to reduce the amount of energy necessary for thermal regulation. Perhaps this mechanism also occurs in sheep.

The greater HP in the CA animals (Table 2), as compared to the WA animals, is to be expected, as more body heat is lost in colder environments due to the increased difference between body temperature and ambient temperature. A fully-fleeced sheep would most likely not increase its HP at 2°C, whereas shearing effectively exposes the sheep to cold stress at that temperature. A shorn sheep at 2°C is equivalent to a fully-fleeced sheep at much lower temperatures (e.g. -20°C). Christopherson and Young (1986) have estimated that the lower critical temperature of an adult shorn sheep is approximately 25°C. At temperatures below this, the sheep must increase its HP in order to maintain body temperature.

The values for HP determined in the WA and CA animals are similar to the results of other workers (Graham and Christopherson 1981; Graham et al. 1981). The CA animals' preinfusion HP was comparable to the level achieved following E infusion in CA sheep in the cold by Graham

and Christopherson (1981), and was similar to the results of Christopherson et al. (1978) and Tsuda et al. (1979, 1984).

Shivering thermogenesis is primarily controlled by the autonomic system and mediated by the somatic nervous system (Webster 1974a). During prolonged cold exposure, adaptive changes are made such that the rate of NST increases relative to the rate of shivering thermogenesis, since shivering thermogenesis is energetically more wasteful over long periods. Thus, there is generally a reduction of shivering observed in CA animals (Sasaki and Weekes 1986). Severe cold (-30°C) has been found to induce a higher HP (maximum thermogenesis) in CA than in WA animals (Webster et al. 1969a).

At thermoneutral temperatures, WA and CA animals are not observed to shiver, however, the CA animals' HP is still above that in WA animals (as will be discussed in Experiment Two). This indicates that there may be some adaptive increase in resting metabolic rate during prolonged cold exposure, facilitated by an enhanced capacity to increase metabolism (Webster et al. 1969a). The animals in Experiment One were acclimated and exposed to chronic cold during measurements of HP, whereas the animals in Experiment Two were only acclimated to chronic cold. The CA animals in Experiment One had a greater HP (Table 2) than the CA animals in Experiment Two (Table 5). This difference between the two groups may be due to the response to immediate exposure to cold. Webster et al. (1969b) investigated the role of catecholamines in HP in WA and CA sheep, and found that NE had an insignificant effect on changing HP. He also stated that NE does not induce NST, however, he did not come to the same conclusion for E. Catecholamines could play a

role in the increase in cold-acclimated NST by increasing the rate of substrate cycling (Challiss et al. 1984a), or by increasing the rate of Na^+,K^+-ATPases (Swann et al. 1981).

Various sources of energy are used by the animal to support an increase in HP during cold exposure, in order to maintain body temperature. Fat, protein, and carbohydrate from body stores and food supplies are used to support the increase HP in the cold. Feed intake normally increases during chronic cold exposure (Sasaki and Weekes, 1986), however if feed intake is not adequate to maintain body weight, as well as HP for maintenance of body temperature, then body condition may decrease during cold exposure. In this study, both the WA and CA animals were fed at levels adequate to maintain body weight and body temperature. The concentration of substrates and by-products used for HP were measured in plasma directly, to see if the β-adrenergic antagonists had any effects on these parameters.

The glycerol, glucose, PUN and some of the amino acid concentrations measured in the CA animals were significantly greater than those measured in the WA animals, indicating that multiple energy sources were being mobilized to a greater degree in the cold (Tables 2 and 3). Perhaps some, or all of these sources were being mobilized as fuel, for the maintenance of body temperature. Apparently, adipose tissue was the greatest source of energy substrate, as the CA animals' average glycerol level was twice as great as that in the WA animals, whereas the change in glucose (17%), valine (88%), isoleucine (34%), and leucine (75%) concentrations was much less (Table 2).

Plasma glycerol was measured because it is a better indicator of lipolysis than FFA levels. When glycerol and FFA's are separated from TAG during lipolysis, the FFA's can be reincorporated into new TAG, at the same site of mobilization. However, the free glycerol is not reincorporated, rather it is transported to the liver and kidney for gluconeogenesis, to produce more glucose, or to be stored as glycogen. Glycerol-phosphate is formed de novo at the adipocyte before it is incorporated into the TAG (Newsholme and Leech, 1983, Newsholme, 1984). Since Table 2 indicates that plasma glycerol levels were greater in CA versus WA sheep, adipose tissue stores must have been used to a greater extent for the metabolic demands in the cold. The WA preinfusion value obtained for plasma glycerol is similar to the results of Thompson et al. (1975) from 21-h fasted rams. However, the CA group's glycerol LS mean was slightly lower than that measured during acute cold exposure of rams by Thompson et al. (1975).

Plasma glucose concentration was significantly higher in the CA animals versus the WA animals, which implies increased mobilization as a response to cold exposure. This gives evidence for the stress effects of chronic cold exposure. During chronic cold stress, plasma E and NE concentrations are elevated (Christopherson et al. 1978; Graham et al. 1981). These hormones function to mobilize glucose and keep supplies of glucose at a high level especially for tissues which have elevated metabolism to maintain body temperature during cold stress.

The plasma glucose levels are similar to the results of Christopherson et al. (1978) and Early et al. (1990), who used mature wethers of approximately the same body weight as used in this study.

The glucose values obtained by Thompson et al. (1978b) from fasted thermoneutral and cold-exposed sheep are also similar to the results in Table 2, even though Thompson et al. (1978b) acutely exposed the sheep to cold. Blood samples which are not promptly chilled, centrifuged and frozen may give erroneously low plasma glucose levels, due to glycolysis by the red blood cells. This should not have been a problem in this experiment, since the samples were immediately put on ice and centrifuged within 15 minutes of sampling.

Plasma urea nitrogen levels were measured as an indication of amino acid deamination to serve either as substrates for oxidation in the liver, or as precursors for gluconeogenesis. Amino acids from muscle are transported to the liver, and are degraded to form their respective α-keto acids and ammonia. The ammonia is then converted to urea, which is exported for excretion via the kidneys, or recycled to the rumen via the saliva or across the epithelium of the rumen. In this way, PUN gives an indirect indication of protein mobilization and catabolism (Swenson 1984). Amino acids can be mobilized from various sources, quantitatively the main one being muscle, because of the large total mass of the tissue. Scott et al. (1992) showed that protein synthesis in skeletal muscle is lower in cold-adapted calves, but that gastrointestinal tissue, which has a large amount of labile protein, was not affected by cold adaptation. However, phenylalanine flux was increased in the calves, which indicates that if less amino acids were being incorporated into protein stores, perhaps they were being incorporated into glucose, and therefore, oxidized.

As Table 2 indicates, PUN levels were higher in CA animals than in WA animals. This indicates that protein turnover may be increased in the cold, but also perhaps that amino acids are catabolized (i.e. oxidized) to a greater extent in the cold. Perhaps the degree of amino acid taken up from the diet is increased in the cold such that more amino acids would be available to the liver for conversion to other energy substrates. Past studies have indicated that apparent availability of amino acids from the small intestine, as well as gastrointestinal tract amino acid metabolism, may be increased relative to other substrates in cold-exposed sheep (Kelly and Christopherson 1989; Kelly et al. 1989). The greater availability of amino acids due to the higher feeding level in the CA animals cannot be ruled out as a cause for greater amino acid oxidation.

Plasma amino acids were measured as another indication of protein metabolism. Specific glucogenic amino acids were evaluated, in order to determine if there were effects of cold exposure and β-adrenergic antagonists on their concentration, and, by implication, amino acid use for gluconeogenesis. The major glucogenic amino acids exported from muscle are alanine and glutamine (Newsholme and Leech 1983). Table 3 indicates that plasma levels of alanine and glutamine were lower in the CA animals than in the WA animals. This either indicates that mobilization of these glucogenic precursors is reduced in CA animals, or that uptake of these amino acids by the liver is increased. To make an accurate assessment of what is occurring, amino acid turnover rates (e.g. by using radioactively-labelled amino acids) should be examined. Early et al. (1990) also demonstrated lower plasma alanine

concentrations in CA sheep. In addition, they found that the percentage of alanine converted to glucose increased in CA sheep, suggesting increased amino acid catabolism in the cold.

The amino acid concentrations measured in the WA group were similar to the results from a mature fed wether, reported by Sedgwick et al. (1991). Any discrepancies between their findings and those presented here may be due to the fact that Sedgwick et al. (1991) only used one sheep. In addition, their sheep was fed, compared to the fasted ones used in this experiment. The concentrations of all amino acids measured, except glutamate and total amino acids, were significantly different in the CA animals versus the WA animals. Kelly (1987) measured mesenteric artery amino acid levels in both WA and CA ewes and found that the amino acid concentrations in CA ewes were larger than those in WA ewes. However these animals were fed, and the concentrations were much greater than the values presented here. The WA and CA values for alanine are similar to those reported by Early et al. (1990) in six mature wethers, however those animals were fed hourly. As for PUN, some amino acid concentrations in the CA animals may have been elevated simply due to the higher feeding level compared to the WA animals.

Effects of β-Adrenergic Antagonist Infusion. The β_1- and β_2-antagonists were used to determine if there are differential effects of the blockade of each β-adrenoceptor on HP and the levels of energy substrates in the plasma. It was expected that blockade of the β_2-adrenoceptors may have resulted in increased emphasis on protein relative to fat catabolism,

since treatment of growing animals with β_2-agonists favours protein accretion (Thornton et al. 1985; Warriss et al. 1989). Although β_1-adrenoceptors are not found on myocytes, yet are found on adipocytes, β_2-adrenoceptors are found on cells which affect both protein and fat metabolism, namely myocytes and presynaptic sympathetic neurons innervating adipocytes (McDowell and Annison 1991).

Neither β-antagonist had significant effects on the WA animals' HP, yet both antagonists had small effects on the CA animals' HP. The effects of these antagonists on the CA animals' HP were not statistically significant when compared to the effects of saline infusion, but the LS means were numerically different. Perhaps there was too much individual animal variation to reveal significant trends. Other possible explanations could be that the animals were not exposed to a low enough temperature such that they would be completely dependent on all of the thermogenic processes available. Perhaps a larger degree of antagonism was necessary to cause a substantial change in HP at 2°C.

Figure 21 shows the regression curves for the response of HP to ICI 118,551. In the WA group, the greatest decrease in HP was seen at the high dose, and the smallest decrease at the medium dose of antagonist. By contrast, in the CA group, the greatest decrease in HP was observed at the medium dose, and the smallest decrease at the high dose of antagonist. This indicates that the greatest difference in response between the WA and CA animals occurred at the B2M dose. In spite of the fact that there is variability among the animals, and the CA curve only approaches significance, there appears to be a different pattern in the warm and the cold. In addition, the effect of the

antagonist approached 15% of the increase due to cold, which could have significant biological consequences. If higher doses of antagonist were used, perhaps it would be seen that the WA group had a maximum response at the high dose used in this study, which decreased after increasing the dose further. This would indicate that the CA group was more responsive to a lower dose of ICI 118,551 than the WA group. Collectively these results do not provide strong evidence that catecholamines are responsible for the cold-induced increase in HP, however, there is evidence that they may have played a minor role.

Only the B1L dose significantly decreased plasma glycerol levels in the CA animals, when compared with saline infusion. It is expected that metoprolol would decrease plasma glycerol, by blocking the β_1-adrenoceptors on the adipocyte cell membrane, which are responsible for activating lipolysis in adipocytes, following catecholamine binding. Thus, decreased lipolysis would be indicated by a decrease in plasma glycerol levels. Perhaps at high doses, metoprolol was showing evidence of agonist-like activity. The significant decrease in plasma glycerol at the B1L dose may not be biologically significant with respect to its effects on HP, because HP was not significantly altered by the β_1-antagonist. That is, the decrease in plasma glycerol did not correlate to a decrease in HP or an increase in plasma amino acids. Perhaps the optimum dose of metoprolol which produces the maximum response has yet to be determined.

From the polynomial regression analysis, it was determined that cubic equations best fit the data for the effects of metoprolol on plasma glycerol (Figure 22). The curve for the CA group indicates that

the maximum effective dose may have been one not actually used in this experiment. The low correlation coefficient (r^2) values indicate that there are large variations among the individual points, so these curves are not very precise. These curves may not be biologically meaningful because there was not a trend or a consistent response to increasing or decreasing dose detected.

Plasma FFA concentration has been shown to increase during chronic cold exposure (Christensen et al. 1990). In addition, turnover and contribution rates of glycerol to newly formed glucose and glycerol and FFA's to total HP were found to increase in sheep exposed to 0°C for 4 d (Tsuda et al. 1979, 1984). Likewise, NE levels have also been shown to be greater in chronically cold-exposed sheep (Christopherson et al. 1978; Graham et al. 1981), which implies that NE secretion is increased or its rate of degradation is decreased. Norepinephrine released from presynaptic sympathetic nerve endings innervating adipocytes, binds to the adipocyte β_1-adrenoceptors, creating an increase in adenylate cyclase activity, which then causes an enzyme cascade which ultimately results in increased lipolysis (Vernon and Sasaki 1991). In order to decrease glycerol release during lipolysis, the β_1-antagonist must have been blocking the adipocyte β_1-adrenoceptor. This would prevent NE from binding and stimulating the β_1-adrenoceptors.

Both the LS means in Figure 4 and the linear regressions in Figure 23 indicate that ICI 118,551 had significant effects on plasma glycerol in the CA animals. Plasma glycerol, measured as an index of lipolysis, significantly decreased at each level of β_2-antagonist used. The β_2-antagonist could have been causing a decrease in plasma glycerol by

blocking the presynaptic β_2-adrenoceptor on the sympathetic nerves innervating the adipocytes. Since stimulation of these β_2-adrenoceptors stimulates NE synthesis and release, blockade of this receptor would be expected to decrease NE synthesis and release, thereby decreasing stimulation of the adipocytes by NE, and thus decreasing lipolysis (the β_2-blocker did not reduce plasma NE in Experiment Two, but this may have been due to insufficient sympathetic stimulation).

With increasing dose of ICI 118,551, a larger decrease in plasma glycerol was indicated. These results indicate that the doses of β_2-antagonist used were more effective at decreasing plasma glycerol than the doses of β_1-antagonist used. It cannot be concluded that the β_2-antagonist was more effective in general than the β_1-antagonist, because it is possible that there is an optimum dose of metoprolol still to be found for sheep, and perhaps that optimum dose of metoprolol will decrease glycerol even more than the B2H dose. However, it would be of interest to determine if even greater doses of ICI 118,551 would further decrease glycerol, and if perhaps at these higher doses, a significant decrease in HP would be observed. Perhaps there is a difference in the affinity of each receptor for its respective antagonist, such that the β_2-receptor has a greater affinity for ICI 118,551, than the β_1-receptor has for metoprolol.

Since glycerol levels were significantly affected by each level of ICI 118,551 used, whereas amino acid levels were only significantly affected by the medium dose, perhaps the presynaptic β_2-adrenoceptors have a lower threshold for stimulation of the intracellular activities than the receptors on the myocyte membrane. This could mean that a

larger dose of β_2-antagonist is required to significantly alter the intracellular processes of muscle cells, which could then be indirectly assessed by plasma amino acid levels.

β_2-agonists, also known as repartitioning agents, are believed to increase protein accretion, while decreasing fat stores (Thornton et al. 1985; Warriss et al. 1989). Their mechanisms of action have never been completely postulated or summarized. Obviously β_2-agonists may simulate the β_2-adrenoceptor-mediated effects of E, and therefore may give some indication as to how E may affect lipid and protein turnover.

As has already been stated, β_2-adrenoceptors are present on the presynaptic sympathetic neurons innervating adipose tissue. Stimulation of the receptors, which occurs with the use of β_2-agonists and E, results in increased lipolysis. This can be measured indirectly by monitoring plasma glycerol levels. Logically, β_2-antagonists would inhibit this process. This is corroborated by the results in Figures 2 and 4, where there were decreases in HP and significant decreases in plasma glycerol concentrations, in both the WA and CA groups, during ICI 118,551 infusion.

It is also evident that inhibition of the presynaptic β_2-adrenoceptor was more effective, at certain doses, at decreasing plasma glycerol, than inhibition of the adipocyte β_1-adrenoceptors. This information correlates to a mechanism for decreased fat in carcasses of animals treated chronically with β_2-agonists. Thornton et al. (1985) also suggests that clenbuterol, a β_2-agonist, decreased rates of lipogenesis and increased rates of lipolysis, and therefore the β_2-agonist induced an increase in the rate of the TAG/FFA substrate cycle.

By analogy, the elevated E levels in the cold may serve to increase the rates of this substrate cycle. The use of the medium dose of β_2-antagonist decreased the rate of this cycle, which also correlated to a decrease in whole-body HP.

In Experiment One, the decrease in glycerol concentration with increasing dose of ICI 118,551 was not associated with a decrease in HP at the highest dose. Since HP at the B2H dose was not significantly different from saline, yet glycerol decreased, this implies that (1) the substrates for HP were being switched at the high dose, or (2) the β_2-antagonist was exhibiting agonist effects on other tissues at the high dose. The two possible substrates for the body to switch to are protein and carbohydrate. Carbohydrate use for HP was not directly assessed in this study, however, plasma glucose concentrations were not significantly affected by β-antagonist infusion. Plasma amino acid and PUN levels were determined to assess possible changes in amino acid utilization, however these levels were not significantly affected by the B2H dose.

The other possibility is that the animals could not support HP by using adipose tissue as a substrate, due to β-blockade of lipolysis, so the use of other substrates increased in order to maintain HP. The plasma glycerol concentration was decreased by approximately 93% of the preinfusion difference between the WA and CA animals following B2H infusion. Other possible energy sources for the animals to use include glycogen stores in liver and muscle and volatile fatty acids produced from fermentation in the rumen (Thompson et al. 1978a; Swenson 1984).

Apparently neither β-blocker had significant effects on the ability of the body to maintain relatively constant plasma glucose levels (Figures 5 and 6). Measuring the concentration of glucose in plasma does not really indicate what is going on at the cellular level, because rates of glucose release from glycogen or its synthesis from precursors is not indicated. These results do suggest that the animals were able to maintain normoglycemic conditions during exposure to the different doses of β-antagonists.

Plasma urea nitrogen was measured to assess the extent of hepatic amino acid oxidation before and after β-antagonist infusion (Figures 7 and 8). Neither β-blocker significantly changed PUN levels compared to saline infusion. If amino acid use for gluconeogenesis and oxidation were required, PUN would be expected to increase. However, in this study, since PUN levels were not significantly affected, perhaps there was not a need to compensate for the decrease in lipolysis. It is also possible that hepatic amino acid oxidation increased, but urinary release of urea also increased.

Total amino acids and some specific amino acid concentrations were analysed to assess protein mobilization. Total amino acids were only affected by the B2M infusion, however this effect only approached significance (Figure 10). Plasma alanine and glutamine were analysed because they are the major glucogenic amino acids exported from muscle (Newsholme and Leech 1983). They are also exported from muscle to remove the transaminated nitrogen groups which are released during amino acid metabolism in muscle. Plasma alanine was not significantly affected by either β-blocker. The WA animals' plasma glutamine was

significantly increased by the B1M infusion (Figure 13) and the CA animals' plasma glutamine was significantly increased by the B2M infusion (Figure 14). The increase in glutamine levels in plasma may be caused by several processes. First, branch-chain amino acid (BCAA) metabolism in muscle may be increased, and thus the nitrogen groups are removed and exported via transamination and release of glutamine (and alanine) from muscle. Second, decreased uptake of glutamine by the liver may increase plasma levels. Third, both glutamine release from muscle, and its uptake by the liver could be increased, but release could be greater than uptake, resulting in greater glutamine in plasma. Finally, increased mobilization of protein from all tissues would increase plasma glutamine levels.

If BCAA metabolism in muscle was increased, it would be expected that their levels in plasma would be decreased. However, in Figures 16, 18, and 20, the BCAA levels are increased, at the same dose of β_2-antagonist where the glutamine levels are increased. This is the opposite result that is expected if BCAA metabolism was increased in muscle. The only way to determine if amino acid metabolism and transamination to glutamate (and then subsequently conversion to glutamine) was increased, would be to measure the relative concentration of glutamine as a ratio of the essential amino acids in muscle tissue versus plasma (Thompson, pers. comm.). If the relative concentration of glutamine in plasma was greater than that in muscle, this would indicate that amino acids were being metabolized in muscle, to form glutamine. If the uptake of glutamine by the liver was decreased, this may indicate that the liver does not require the amino acid glucogenic precursors and

that adequate glucose sources were present. There is no evidence to dispute this possibility, since the plasma glucose levels were maintained during the β-antagonist infusions. Since the levels of all of the amino acids measured were increased at the B2M level, then this implies that increased generalized mobilization of protein occurred.

β_2-agonists have also been shown to increase protein accretion in skeletal muscle (Thornton et al. 1985; Warriss et al. 1989). Since E is increased during cold exposure and muscle is a large proportion of body mass, it is implicated that E could be using the protein synthesis and degradation substrate cycle as a mechanism to increase thermogenesis in muscle during cold exposure. However, studies with clenbuterol (Reeds et al. 1986) and cimaterol (Rikhardsson et al. 1991) indicate that these β_2-agonists have little effect on muscle protein biosynthesis and thermogenesis. Protein accretion is then probably achieved through a decrease in protein degradation. In addition, E increases during cold exposure probably also act on this pathway to decrease protein degradation. In a cold winter environment, it would make sense that the body, for example, via increases in E concentration, would shut down protein degradation for use in oxidation, and amplify the lipolysis pathway, which provides more energy from oxidation than protein, without the problem of nitrogen excretion. These findings help to explain the results from the use of ICI 118,551. At the B2M dose, the blocker would stop the effects of E (a β-agonist), therefore protein degradation would not be inhibited. This is indirectly indicated by an increase in the plasma amino acids at the B2M dose (Figures 14, 16, 18, and 20). The high dose of ICI 118,551 may have acted as an agonist on myocytes and

therefore protein degradation was inhibited, and in turn, plasma amino acid levels decreased. Perhaps this is the reason for the quadratic appearance of the glutamine response in Figure 25.

The results indicate that apart from research purposes, although both antagonists appeared to decrease a product of lipolysis, i.e. glycerol, there would be no beneficial practical use of these antagonists in meat-producing animals, unless they improved energy conservation. The results imply that β_2-adrenoceptor-mediated processes may be more important (i.e. more sensitive) in controlling lipolysis, as compared to β_1-adrenoceptor-mediated processes. This may be too much of an assumption from this experiment because a wider dose range with more replicates is necessary, to truly determine the optimum dose required to obtain the maximal response. Perhaps the dose range was too large, and doses less than 2.5 $\mu g\ kg^{-1}\ min^{-1}$ should be investigated. More replicates would also help to decrease the variance of the means. It would be beneficial to perform a crossover study, where the WA and CA animals would switch environments with each other, and then the same infusions performed again. This would double the sample size and perhaps decrease the variability of the measurements.

EXPERIMENT TWO

The infusion rates of metoprolol, ICI 118,551, and E used for Experiment Two were sufficient to cause significant changes in several of the parameters measured, relative to saline infusion. The changes in these parameters were all similar to those reported for Experiment One.

The 1 h period prior to the beginning of the infusions was probably sufficient for the CA sheep to adjust to the warm environment since Webster (1966) found that shorn sheep can more rapidly adjust to changes in ambient temperature than fully-fleeced sheep. Even though Webster (1966) looked at the effects of cold exposure, the principle he suggests should apply to increases in ambient temperature. Heat production following E infusion was similar to the HP of outdoor sheep exposed to -30°C (Webster et al. 1969a).

The NE pre- and postinfusion concentrations, and the E preinfusion concentrations were within the ranges reported by other workers (Christopherson et al. 1978; Thompson et al. 1978c; Graham et al. 1981), for both the WA and CA groups. Following the infusions that included E, the plasma E concentrations were higher than those reported for both WA and CA sheep (Christopherson et al. 1978; Thompson et al. 1978c; Graham et al. 1981), and also higher than those reported for the CA sheep in 2°C in Experiment One. Other studies which also looked at the effects of E infusion on CA sheep did not measure the change in plasma E and NE levels (Graham et al. 1980; Graham and Christopherson 1981; Bolton and Weekes 1986). This makes it difficult to assess whether or not the postinfusion catecholamine levels measured were within physiological ranges for sheep. The dose of E used was chosen due to its use in past experiments (Graham et al. 1980; Graham and Christopherson 1981), where this dose cause a substantial increase in HP. Bolton and Weekes (1986) proposed that E doses of 0.05 and 0.25 $\mu g\ kg^{-1}\ min^{-1}$ would result in increases in plasma FFA and glucose to concentrations seen in animals

under stress, while infusion of E at higher doses, e.g. 0.67 $\mu g\ kg^{-1}\ min^{-1}$, would have pharmacological effects.

The largest change in HP, which occurred following E infusion, was much larger than that reported by Christopherson et al. (1978), as well as by Graham and Christopherson (1981) when comparing the HP after infusion of the same dose of E. However, Graham and Christopherson took an average HP over the infusion period, so lower HP values at the beginning of the infusion could have decreased the overall HP, compared to this study.

From the largest change in glycerol concentration which is shown in Figure 27, the glycerol concentration of the sheep reached a maximum of about 2.4 mg dL^{-1} during E infusion. This level is twice that measured by Thompson et al. (1975) in 6 h and 21 h fed sheep exposed to 0.5 to 4°C for 3 to 4 h. However, those researchers only looked at the effects of acute cold exposure.

The change in glucose concentration following E infusion was much larger than concentrations reported for CA animals without E infusion (Horton 1981; Christensen et al. 1990; Early et al. 1990). This indicates the dose of E was successful in elevating plasma glucose. The change in glucose in the CA sheep was much larger than that reported by Bolton and Weekes (1986), using an E dose rate of 0.05 $\mu g\ kg^{-1}\ min^{-1}$ in WA animals, which was six time less than the dose rate in this study.

The PUN and amino acid maximums reached are comparable to the concentrations found in the plasma of fed sheep (Thompson et al. 1978b; Horton 1981; Early et al. 1990), although the PUN levels are slightly higher than reported values. The difficulty here is that there is very

little data on plasma levels of different substrates in CA sheep. Therefore, interpretations made are entirely on the assumption that the animals are considered "normal" for their particular environment.

The EB1 infusion, which included metoprolol, significantly decreased glycerol levels, when compared to E infusion in both WA and CA animals. However, the change in glycerol from EB1 infusion was still greater than saline, so metoprolol did not completely remove the effects of E. The other parameter changes measured (HP, glucose, PUN, amino acids) were not significantly different from E, so metoprolol did not remove the effects of E on these parameters.

The EB2 infusion, which included ICI 118,551, significantly decreased HP in the WA animals, compared to the effects of E infusion. The change in the CA animals only approached significance, so perhaps the WA animals were more sensitive to the effects of ICI 118,551 during E infusion, than the CA animals. Since the change in glycerol in both WA and CA groups after EB2 infusion was not significantly different from saline, but was significantly different from E, perhaps ICI 118,551 was successful in removing the effects of E infusion. This response to β_2-blocker infusion correlates with the mechanism of action of β_2-agonists and E described previously in Experiment One. A decrease in glucose concentration was also seen in the CA animals following EB2 infusion, however, since the change was not significantly different from E infusion, ICI 118,551 did not completely remove the effects of E infusion. In the WA animals, ICI 118,551 did not significantly change the effects of E infusion on glucose concentration.

The significant difference in PUN between E and EB2 infusions in the WA group is of doubtful importance since neither infusion caused a change significantly different from saline. This is also true for these infusions and their effects on valine. However, leucine and isoleucine concentrations did increase with EB2, in the CA animals. The results for leucine and isoleucine also support the mechanism of action of β_2-agonists and E described previously for Experiment One.

For both the WA and CA animals, EB2 was more effective at decreasing glycerol than EB1. Perhaps this indicates that the β_2-blocker, at the dose used, was more competitive than the β_1-blocker because measured E levels during the EB2 infusion were greater than during the EB1 infusion. In addition, perhaps the effects of β_2-adrenoceptor stimulation on the presynaptic neuron of adipocytes is more sensitive to blockade than the β_1-adrenoceptors on adipose tissue.

The epinephrine data is very difficult to interpret (Figure 36). It is expected that the CA animals would have greater E levels overall than the WA animals, but it was unknown how each group would respond to E infusion. Perhaps tissue uptake or clearance rates of E may be slower in the CA animals, as the change in E concentrations in the CA animals were significantly greater than for the WA animals. It might be expected that the E concentrations in all of the infusions that included E would be similar because of the similar dose of E used. However, this was not evident from the results. What makes this data difficult to interpret is that even though the differences were found to be statistically significant, conclusions must be made with caution, since only three animals were used. Since these animals had never been

previously exposed to exogenous E, perhaps the response to the first exposure of E was different from the subsequent exposures. There has not been any evidence that adrenergic antagonists competitively inhibit E binding to the extent that free plasma E levels (i.e. not bound to receptors) would increase. However, this could be a possible explanation to the significant difference shown between the WA animals' EB2 and E infusions.

It is not surprising that the NE concentration did not increase following E infusion, even though E can bind to the presynaptic β_2-adrenoceptors to cause an increase in NE synthesis and release (McDowell and Annison 1991). Perhaps there has to be a sympathetic drive present, i.e. an increase in NE synthesis and secretion, in order for E to modulate NE release (as described previously in Experiment One). Since both groups of animals were in a warm environment during the infusions, there was not a need to increase sympathetic stimulation. In addition, plasma NE concentrations may not be a true reflection of the degree of sympathetic stimulation, since NE is quickly degraded by enzymes at the site of release (Hadley 1984).

β-adrenergic antagonists were used to separate the specific β-adrenoceptors (that are most responsive to E), and therefore, the response to catecholamines, from other hormones. The results of Experiment One suggest that catecholamines do play a role in the increase in cold-induced HP, however further studies should be done using lower temperatures of acclimation and exposure. If an animal could be forced to its summit metabolism, perhaps responses to changes in substrate concentration could be amplified. In this study, β-

antagonist infusion did decrease the amount of plasma glycerol, one of the energy substrates for cold-induced thermogenesis, however this decrease did not correlate to a significant change in HP. Perhaps the individual variation between the animals was too large. It is necessary to obtain more replications and it would be beneficial to perform a cross-over experiment, switching the animal groups' environments.

Since the greatest change in glycerol and some of the amino acids was observed with the β_2-antagonist, perhaps the effects of stimulating the β_2-adrenoceptor are more important in regulating the energy substrates used for HP. In addition, the greatest increases in amino acid concentrations were observed at the medium dose of ICI 118,551. It is possible that using this drug at this dose changes the body's use of adipose tissue to protein. Further experiments could include larger doses of both antagonists, with smaller increments between doses, to see if further changes in the parameters could be detected. It would also be interesting to discover if the concentration and affinity of the β-adrenoceptors are different between WA and CA animals, and if the relative concentrations of each receptor subtype changes with chronic cold exposure. If the second experiment, were repeated, it may be necessary to monitor the plasma metabolite changes at more frequent intervals, to see if there are initial changes due to E infusion, which are later masked or reversed by the action of the β-antagonists.

Heat production can be decreased by the use of β-antagonists, even if the decreases in this study only approached significance. The decreases obtained with both β-antagonists appeared to be biologically significant because they represented between 13 to 15% of the increment

in preinfusion HP between the WA and CA animals. The β-antagonists had significant effects on the plasma glycerol and amino acid concentrations, implying that catecholamines, via β-adrenoceptors, can alter lipid and protein mobilization. There was a calorigenic effect of E infusion. Collectively this evidence supports a role for catecholamines in contributing to the cold-induced increase in HP.

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Appendix Table 1: The effects of various 3-h infusions of saline, metoprolol (B1), and ICI 118,551 (B2) on rectal temperature changes in wethers (warm = WA = 26°C; cold = CA = 2°C) in Experiment One (n = 6 unless otherwise noted by a superscript; all comparisons $P>0.05$).

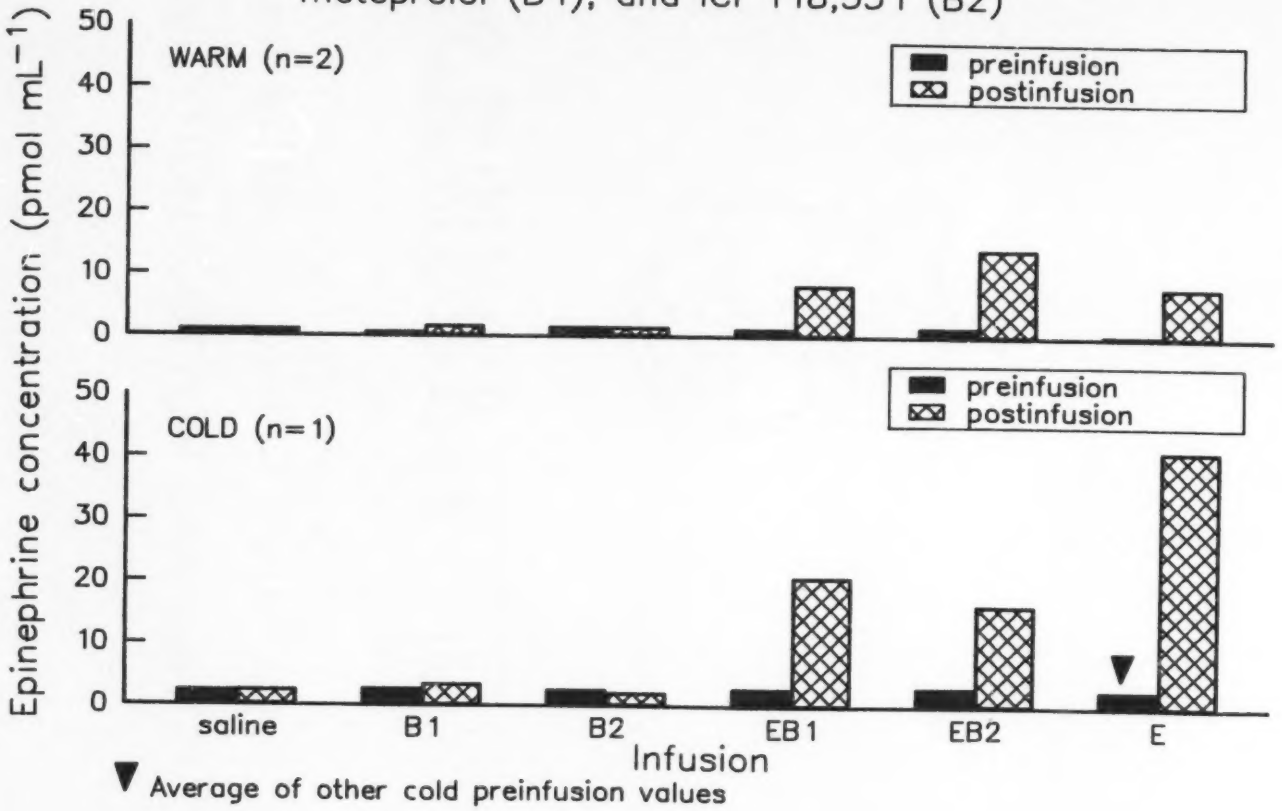
Infusion[a]	WA animals (± SEM)	CA animals (± SEM)
saline (B1 series)	0.2 (± 0.26)[b]	0.7 (± 0.20)
B1L	0.3 (± 0.20)	0.8 (± 0.20)
B1M	0.6 (± 0.20)	1.2 (± 0.20)
B1H	0.3 (± 0.20)	0.7 (± 0.23)[c]
saline (B2 series)	0.0 (± 0.22)[b]	0.7 (± 0.17)
B2L	0.6 (± 0.17)	0.8 (± 0.20)[c]
B2M	0.2 (± 0.17)	0.6 (± 0.20)[c]
B2H	0.5 (± 0.17)	1.1 (± 0.17)

[a] B1L, B2L = 2.5 $\mu g\ kg^{-1}\ min^{-1}$; B1M, B2M = 5.0 $\mu g\ kg^{-1}\ min^{-1}$; B1H, B2H = 10.0 $\mu g\ kg^{-1}\ min^{-1}$

[b] n = 4

[c] n = 5

Appendix Figure 1: Plasma epinephrine levels in warm– and cold–acclimated wethers before and after combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)



Appendix Figure 2: Plasma norepinephrine levels in warm– and cold–acclimated wethers before and after combination infusions of epinephrine (E), metoprolol (B1), and ICI 118,551 (B2)